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An investigation of Warner-Bratzler shear gradients in two beef muscles and an exploration of muscle specific treatments for enhancement of instrumental tenderness

by

Jennifer Anne Marie Janz

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Department of Agricultural, Food and Nutritional Science

Edmonton, Alberta
Spring 2004

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Abstract

Tenderness is a critically important quality trait and its lack of consistency, particularly in beef, is a challenge to the meat industry worldwide. More information about individual muscles is needed to bolster attempts at muscle specific treatments to enhance tenderness, thus the initial objective of this work was to develop a method for mapping Warner-Bratzler shear force, taking into account the shortcomings of previously reported methods. A method was, therefore, developed to thoroughly map shear gradients in the *Longissimus thoracis et lumborum* (LTL) and the *Semitendinosus* (ST).

Significant shear differences and substantial variability were observed in all dimensions of the LTL, but were particularly evident in the medial-lateral plane, possibly due to variable tension development across the muscle during conventional carcass suspension. Mapping of both shear and sarcomere length showed a reduction in this variability following altered carcass suspension and demonstrated the effectiveness of the mapping technique. While shear values were greater in the distal and proximal extremities and superficial locations within the ST, this muscle displayed less variability than the LTL. Furthermore, shear values were significantly reduced by the removal of epimysial connective tissue. Modified chilling and extended ageing were also applied to a variety of beef muscles. Results indicated a variable response in the selected muscles, thus emphasizing the need to implement muscle specific tenderness enhancement strategies.

Since the instrumental tenderness of the ST was not amenable to change using simpler methods, the final objective was to determine the effectiveness of various injection treatments for reducing shear values. Preliminary work demonstrated the

successful application of an injected enzyme complex. Further investigation showed that the inclusion of sodium tripolyphosphate in the brine improved the tenderization response. Variable results, however, were a clear indication of the need for further refinement of the injection treatments for use under specific cooking conditions.

Complete knowledge of the inherent characteristics and processing attributes of each component of the carcass musculature would provide a solid starting point from which muscle specific tenderness interventions could be developed. The application of muscle appropriate treatments is essential for meeting the product quality goals of the beef industry.

Dedication

A long time ago my Dad told me a story about a man who went about consulting the
wisest men in the world in order to find the meaning of life
and what he found was that "There is no free lunch"

My Dad also told me that the only way to even begin to achieve your goals
is to clearly state to the world what you intend to accomplish

So anyway Dad, I was listening
and I have tried to keep these words of wisdom in mind
as I have worked my way through school

This dissertation is dedicated to my Dad
(I think he always wanted me to be a doctor!)

and

To my boys
Who continue to take life's greatest leaps with me

Are you ready?

Here we go again!

Acknowledgements

Once again, my supervisor, Dr. Mick Price, has steered me towards program completion, and for his wise and gentle guidance I am grateful. I would also like to extend sincere thanks to the members of my supervisory committee Dr. Jennifer Aalhus and Dr. Mike Dugan (Agriculture and Agri-Food Canada Lacombe Research Centre), and Dr. Wendy Wismer (Department of Agricultural, Food and Nutritional Science, University of Alberta) all of whom devoted time and guidance to ensure my work was positive and progressive. I am also appreciative of the time and effort donated by the members of my examining committee, Dr. Ted Putman (Department of Physical Education and Recreation, University of Alberta) and Dr. Chris Calkins (Animal Science Department, University of Nebraska) and the committee chair, Dr. Edward Bork (Department of Agricultural, Food and Nutritional Science, University of Alberta).

During the course of my PhD work, I received, and I am grateful for, financial support from both the Natural Sciences and Engineering Research Council of Canada (NSERC) and the University of Alberta in the form of a Post Graduate Scholarship and a Dissertation Fellowship. Despite a serious industry crisis, the Beef Cattle Research Council (BCRC) saw fit to provide funding for the final phase of my work; personal thanks to Rob McNabb of the Canadian Cattlemen's Association for making that happen. I would like to acknowledge Phyllis Shand at the University of Saskatchewan (U of S) for administering the BCRC funds and for her hospitality during my time in Saskatoon.

The administration at the Agriculture and Agri-Food Canada Lacombe Research Centre was kind enough to permit my residence during the majority of my PhD time and the list of thanks owed to the staff is extensive. Thanks go to Bill Starr and Ken Grimson from the Beef Unit for animal care and transportation; Don Brereton and Chuck Pimm from the Meats Centre for slaughter and processing; and Rhona Thacker and Dave Best for laboratory consultation advice. Ivy Larsen, Fran Costello, Kristy Landry, and Dave Rolland also donated time to help in the lab during periods of intense work. Mike Dugan was always available on a moment's notice to bounce ideas around, and Bethany Uttaro joined me in some serious cogitation. The assistance of Kathy Moore at the Lacombe Research Centre library was indispensable. Of all the people in Lacombe, Jennifer Aalhus took on the ultimate responsibility for "hosting" me at the Research Centre, and

in facilitating my extended visit, provided access to lab space and resources, as well as an office well beyond even the wildest grad student dreams. I am extremely grateful for the privilege of having been able to conduct my work in such an environment. Thanks to Jody Forslund and Francine Hodder in the Department of Agricultural, Food and Nutritional Science at the University of Alberta for taking care of the administrative details that kept my academic program on track. I am also grateful to John Basarab at Alberta Agriculture, Food and Rural Development for his statistical input and advice on experimental design. Thanks to Egon Skovmose, at Danlac in Airdrie, AB, who was extremely helpful in facilitating a smooth delivery of the enzyme product from its New Zealand origin. Special thanks are extended to Zeb Pietrasik, post-doctoral fellow at U of S, for sharing ideas and the work load during the last months of experimentation. It was a real pleasure to work with you all.

Gratitude must also be bestowed upon my dear friends, Dave "LD" Rolland, who kept me thinking about the world at large during our routine digressions into current issues, Canadian politics, and cheese varieties, and the frequently extemporaneous and often tangential Robin MacInnis, both of whom joined me in moments of near defenestration and others of addlepation during my work, particularly, in these final months.

Sincerely,
Jen

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Chapter One

General Introduction and Literature Review

1.1. Introduction

Almost every recent publication concerning beef tenderness is prefaced by a strong statement about a lack of consistency in this trait and/or a serious need to address consumer demands for tender product on a consistent basis, since tenderness is the most important variable for ensuring satisfactory eating quality. The current discussion is no exception. Meat toughness/tenderness and the underlying causes contributing to both the development and variability of this texture continuum are interesting and fundamental issues, and research in this area will be ongoing. Myofibrillar toughness/tenderness is the net result of two opposing processes: toughening during rigor development and subsequent tenderization. Minimizing the former and maximizing the latter are worldwide research goals (Tarrant 1998). Furthermore, while the contribution of connective tissues has been relegated to "background toughness" (Bailey 1972; Tarrant 1998; Taylor 2003), connective tissues are present in varying proportion in every muscle due to the functional nature of the tissue, and their role in the tenderness/toughness issue must be addressed.

Meat colour, flavour, aroma, tenderness, and method of cooking collectively influence consumer acceptance (Morgan et al. 1991), with tenderness being the most important driver of liking (Beef Information Centre 2002). A number of statistics from national beef quality surveys conducted in Canada and the United States, however, indicate a deficiency in meeting consumer expectations, particularly with reference to tenderness. Across a selection of chuck, loin, and round steaks evaluated during the Beef Consumer Satisfaction Benchmark Study (Beef Information Centre 2002), only 68% of consumers were satisfied with tenderness, that is, provided a score of ≥ 7 out of 10. For the inside round (*Semimembranosus*) alone, this value fell to 55%, while striploin (*Longissimus lumborum*) steaks provided 82% of consumers with tenderness satisfaction. In the National Beef Tenderness Survey conducted in 1990 (Morgan et al. 1991), and

based on 50 and 68% confidence levels, 56.4 and 87.2% of eye of round (*Semitendinosus*; ST) steaks were above the range of shear values related to a "slightly tender" rating upon sensory evaluation. When the survey was repeated in 1998 (Brooks et al. 2000), these values showed some improvement (26.6 and 55.9% of ST steaks had shear values outside the 50 and 68% confidence levels for "slightly tender", respectively), but were still high and certainly out of range of the 100% consumer satisfaction goal set by the Canadian beef industry (Beef Information Centre 2002). When consumers were asked to provide a reason for their less than ideal eating experience, tenderness complaints were most prevalent, and 88% of consumers cited product deficiencies as the cause (Beef Information Centre 2002). To ensure an optimal product reaches consumers, then, the largest share of the responsibility is assigned to the beef industry (Beef Information Centre 2002).

These national surveys provide strong indications from consumers about what makes a desirable product. Meat quality research can address these issues by defining the inherent muscle characteristics that might guide specific treatment of individual muscles in order to maximize their most positive qualities and enhance those that require improvement. Furthermore, Tarrant (1998) indicated that the dearth of new meat product development compared to almost any other food sector has led to criticism about a failure of the meat industry to move beyond primary processing, the preparation of cuts no longer compatible with the current lifestyle, and the slow emergence of convenience products. This is the dawn of a period in which branded meat products are gaining popularity in the retail meat case; a period with excellent potential to introduce novel products to consumers. These products may represent nothing more complicated than maximizing the natural characteristics of a muscle with a new set of fabrication specifications, or they may involve more complex further processing to create entirely new products.

1.2. Beef Tenderness Characterization

To identify opportunities for alternative fabrication or processing, a thorough understanding of the inherent characteristics of individual muscles if first required. A sampling of work conducted from the mid-1960s to the present indicates the intention of

many workers to describe beef tenderness trends, primarily in the *Longissimus*, by evaluating Warner-Bratzler shear and sensory attributes along longitudinal and cross sectional planes of the muscle. What characterizes the majority of these reports is the limited number of muscle locations used for testing, along with the underlying assumption that these results could be extrapolated, with reasonably validity, across the entire length and width of the muscle.

Segars et al. (1974) presented the most comprehensive analysis, using the entire length of each muscle of interest, and evaluating both longitudinal and cross sectional trends; however, use of a cyclical compression test that failed to maintain uniform fibre direction made comparison with other studies difficult. Evaluations of longitudinal trends were generally based on three to six steak locations along the muscle (Henrickson and Mjoseth 1964; Jeremiah and Murray 1984; Gariépy et al. 1990; Wheeler et al. 1996), in some cases restricted to only the lumbar portion of the *Longissimus* (Martin et al. 1970; Williams et al. 1983). As a part of their review of 40 different bovine muscles, however, Belew et al. (2003) provided more extensive longitudinal sampling, focussed on several muscles for which this type of evaluation had not previously been conducted (for example, *Psoas major*, *Gluteobiceps*, *Infraspinatus*, *Pectoralis profundus*). In the cross section, reporting of differences in the superficial to deep plane was rare (Smith et al. 1969). Reports tended to focus on medial to lateral differences, most based on an evaluation of two to four core samples across each steak (Cover et al. 1962; Hostetler and Ritchey 1964; Alsmeyer et al. 1965; McBee and Wiles 1967; Hedrick et al. 1968), although some studies made use of as many as five or six cores (Smith et al. 1969; Crouse et al. 1989; Berry 1993). In the wake of these methodologically limited reports, a new wave of beef tenderness characterization research has arrived, and is well defined by three recent reports describing detailed mapping methods, each with a slightly different approach to the concept.

Kerth et al. (2002) used 320 striploins (*Longissimus lumborum*) and sampled one steak from each of the "anterior" and "middle portion" locations. Anterior steaks were aged 7 days, then frozen, while middle portion steaks were aged 14 days. For Warner-Bratzler shear force measurement, cylindrical samples were removed from 15 predetermined locations across each steak, discarding cores that were misshapen or that

contained connective tissue. This group presented means and standard deviations for each sampling location and created surface plots to visually represent the trends. MaxR regression was also employed to determine average predictive value of various core locations.

Zuckerman et al. (2002) also used striploins. Seven muscle pairs were aged 5 days then divided into 2.5 cm steaks and frozen prior to shear mapping analysis. This group chose rectangular shear samples to improve repeatability of sample shape and location. The central area of each steak was avoided due to the potential presence of large amounts of connective tissue, and this region was used as a sampling landmark. Twenty-five predetermined sampling locations were identified with a grid system and shear results were presented as a surface plot. Furthermore, these authors demonstrated the applicability of their mapping method to the evaluation of the shear-reducing capabilities of a hydrodynamic wave pressure treatment.

Reuter et al. (2002) examined muscles of the round (*Semimembranosus*, *Biceps femoris*, *Adductor*, and *Semitendinosus*) from one side of each of 10 animals. All muscles were aged 8 days then frozen prior to their division into steaks. A fishhook was inserted into each steak to maintain a constant and identifiable location throughout the cooking and shearing processes. Steaks from the larger muscles were divided into "zones" with a goal of removing at least six good cylindrical shear samples per zone. Shear values were later averaged within each zone. The ST was divided into 11 steaks with an average calculated across each.

After reviewing the methods employed in each of these mapping studies, a number of shortfalls were apparent. While Kerth et al. (2002) examined muscles from a large number of animals, and reasonably extensive sampling was performed in the muscle cross section, only two longitudinal locations, restricted to the lumbar portion of the *Longissimus*, were studied. Zuckerman et al. (2002) also restricted their study to the lumbar *Longissimus*, but conducted more extensive sampling with a greater number of steaks per muscle and more shear samples per steak than Kerth et al. (2002). Unfortunately, shear values along the length of the muscle section were averaged at each shear measurement location and the opportunity to study longitudinal trends was lost. Reuter et al. (2002) mapped along the length of each of the muscles studied, but averaged

within the cross section by zone or by steak. So, no single study examined all three dimensions of the given muscles, and none preserved anatomical relationships. Furthermore, all studies used aged product that was also frozen at some point prior to shear force evaluation, thus introducing the potential to alter or mask inherent shear characteristics by permitting a period of postmortem proteolysis and a damaging freeze/thaw cycle (Geesink et al. 2001).

While fresh, unaged samples are essential for the establishment of a true baseline for each muscle subjected to a mapping method, this type of detailed examination can be logistically challenging. Zuckerman et al. (2002) reported that sample preparation for the mapping method was more time consuming than traditional techniques, hence their use of a limited number of animals. Preparation became faster, however, as experience with the method increased, with the advantage over earlier methods of yielding more information than a limited selection of cylindrical cores can provide. Shear force "maps" created by deducing trends from voluminous data are infinitely more representative than trends extrapolated from limited locational data. Although mapping techniques may not be practical for use in general treatment comparison studies, they are invaluable for the discovery of inherent trends within various muscles. Data of this type could then be applied to solve current, practical problems through the exploitation of inherent characteristics in the development of muscle specific tenderness enhancing strategies.

1.3. The Case for Muscle Specific Interventions

One important conclusion drawn from the Beef Consumer Satisfaction Benchmark Study (Beef Information Centre 2002) was that tenderness improvement of already tender meat has a minimal impact on consumer satisfaction. A far more effective approach would be to target less than satisfactory products in order to concentrate resources where the greatest improvements can be achieved. Alternatives for selection of target products include the identification, perhaps by some rapid on-line evaluation method, of tough meat that requires a tenderizing treatment, or the application of an intervention to all cuts from tougher portions of the carcass (Beef Information Centre 2002). This is distinct, of course, from the blanket application of a post-fabrication treatment, for example moisture enhancement, to all cuts from the carcass.

Koohmaraie (1996) stated "that there are [*sic*] a variety of methodologies to eliminate the inconsistency of meat tenderness at the consumer level. The question that needs to be addressed is: Why are these technologies not adopted by the industry? It is, perhaps, far more urgent to answer this question rather than it is to develop more technologies." Taking this concern one step further, successful and economical use of these technologies at the industrial level will depend on their appropriate application; that is, their administration to specific muscles in which evidence of the beneficial effects of the particular treatment are expected. Application of a tenderness-enhancing process to an already satisfactory product or to a product that does not respond well to treatment represents a misappropriation of effort and resources.

Amongst the available treatments with demonstrated effectiveness for the amelioration of toughness are ageing, altered carcass suspension, and a variety of modified chilling regimes; however, limitations to the use of each of these processes exist. For example, carcass suspension by the *obturator foramen* or involving the severing of strategic bone and connective tissue locations is effective primarily in the hindquarter muscles, and even amongst those the treatment effect may be variable (Wang et al. 1994; Eikelenboom et al. 1998; Aalhus et al. 1999). Demonstrating the redundancy of tenderizing tender muscles, Jeremiah et al. (1999) evaluated blade tenderization in a number of boneless beef cuts. While the treatment resulted in an impressive 0% of *Longissimus* samples rated tough overall, this was reduced from only 4% initially, in stark contrast to the *Semitendinosus* which showed a reduction from 76% to 32% after treatment. Clearly, it is not reasonable to apply blade tenderization to the *Longissimus*, and while a large reduction in overall toughness of the *Semitendinosus* was observed, other methods could perhaps yield better results for this muscle. Real potential for further processing that includes injection marination or moisture enhancement using phosphate-containing brine was demonstrated in the consumer satisfaction study (Beef Information Centre 2002). The survey of steak cooking methods indicated that only 10% of marinating steaks were prepared correctly, possibly due to consumer time constraints on meal preparation. Inclusion of the marinade in the retail-available products would reduce preparation time. Furthermore, the survey conclusions included a statement regarding the importance of including a tenderizing agent in the marinating process.

Given its poor performance after such harsh mechanical treatment as blade tenderization, the *Semitendinosus* appears to be an ideal subject for this type of post-fabrication, pre-merchandising treatment.

1.4. The Use of Injected Phosphates in Whole Muscle Meat Products

Phosphate injection has been used commercially in chicken for more than 30 years (Grey et al. 1978). The process has since been generally termed "enhancement" since the eating quality of the final product is enhanced (Miller 1998). Retail-ready injected pork products are now commonly available in the United States (Vote et al. 2000) and Canada, particularly in Ontario (personal communication, J. Gariup, July 2002), with some availability in the western provinces (Glaeser et al. 2003). Research reports on the quality enhancing effects of injected phosphate brines in pork are abundant (Table 1.1), and interest in beef, both research (Table 1.2) and industry (Nunes 2000) oriented, is emerging. Currently, only a small proportion of beef cuts is used in further processing, but additional information about the processing characteristics will permit increased utilization of beef, particularly under-valued cuts that may be used for ground product (Boles and Shand 2001), and will improve phosphate injection success.

Much of the benefit of moisture enhancement is reaped from the improved water holding capacity induced by added phosphate (Cannon et al. 1993; Sheard et al. 1999; Brewer et al. 2002). Water holding or binding capacity (WHC) is the ability of meat to retain its natural water and is closely related to the ability of meat to take up additional water in the presence of elevated salt concentration (Offer and Trinick 1983). Water holding capacity has enormous economic importance because it not only influences the amount of product available for sale on a weight basis, but ensures satisfaction with sensory properties such as juiciness, texture, and flavour (Offer and Trinick 1983; Trout 1988; Van Laack 1999). In general, moisture enhancement using a phosphate brine positively affects sensory attributes with few detrimental effects on physical characteristics (Prestat et al. 2002). The WHC-related yield improvements provide a reduction in purge loss (Cannon et al. 1993; Sutton et al. 1997), and a decrease in cooking loss (Brewer et al. 1999; Sheard et al. 1999). Improvements in tenderness and juiciness (Cannon et al. 1993; Brewer et al. 1999) and a reduction in shear values

(Robbins et al. 2002) are commonly reported. Sutton et al. (1997), however, did not find a reduction in shear value following injection treatment, although as expected, sensory tenderness and juiciness scores were improved.

Sodium tripolyphosphate is the phosphate compound most commonly used in injected meat products, often in conjunction with sodium chloride. When used as the sole brine ingredient, phosphate can impart a less preferable (Griffiths and Wilkinson 1978) soapy or sour flavour (Miller 1998; Vote et al. 2000). Not only does salt tend to mask these off flavours (Miller 1998), but its presence also enhances the uptake (Cannon et al. 1993) and performance of injected phosphate brines (Bendall 1954; Offer and Trinick 1983; Trout 1988; Vote et al. 2000).

The basic mode of action of phosphates in meat is the elevation of pH (Trout and Schmidt 1984; Brewer et al. 1999) that promotes a chain of events resulting in increased water holding capacity, and ultimately, disruption and solubilization of the myofibrillar structure (Ellinger 1972). Injected phosphates move meat pH away from its isoelectric point (Ellinger 1972; Miller 1998) causing electrostatic repulsion between myofilament proteins and a resultant increase in myofibrillar spacing that effectively increases the amount of physical space available for water binding (Offer and Trinick 1983). Furthermore, a concomitant increase in salt concentration results in depolymerization of myosin filaments (Offer and Trinick 1983), while phosphates have an ATP-like interaction with actomyosin that dissociates this compound (Bendall 1954) bringing about disruption of the myofibrillar structure, thus increasing tenderness.

Of the various phosphate compounds, pyrophosphate is the simplest of the polyphosphates, containing two phosphate units, and appears to cause the greatest swelling effect (Bendall 1954). Tripolyphosphate is the next most effective of the phosphates, although there is some indication that it is hydrolysed to pyrophosphate by endogenous phosphatases or by the action of myosin ATPase (Offer and Trinick 1983; Xiong 1999). As phosphate chains become larger their effectiveness is progressively reduced due to reduction of ionic strength and pH (Trout and Schmidt 1984).

Commercial injection of beef is not widespread but the process could aid in meeting consumer expectations for consistency and quality of tenderness and juiciness, and further investigation of less tender cuts is warranted (Vote et al. 2000).

1.5. The Use of Enzymes for Meat Tenderization

Enzymes are biocatalysts produced within living cells for the purpose of assisting with specific metabolic processes. The fact that they can be removed from the cellular environment and maintain the ability to perform their functions makes them useful for other applications within food systems (Underkofler 1972). Enzymes lower the activation energy required to promote chemical reactions such that these events can occur under milder conditions (e.g. room temperature, near-neutral pH). Harsh processing conditions (e.g. high temp, high pressure, high acidity), which can in themselves cause unwanted changes or require special equipment, and/or extreme time periods are then avoided (Underkofler 1972). Enzymes occurring naturally in muscle tissue can produce favourable postmortem improvements in tenderness and flavour (Underkofler 1972) over an extended ageing period. The application of exogenous enzymes can increase the rate and extent to which these changes occur.

The use of enzymes in food production is not a new process. Examples such as fermentation, the use of stomach mucosa for cheese clotting (Underkofler 1972), and the application of unripe papaya extract for meat tenderization demonstrate that knowledge of these preparations has been known for centuries (Wang and Maynard 1955; Underkofler 1972; Fawcett and McDowell 1987). While the users were most certainly unaware that enzymes were effecting the desired changes, crude preparations became items of trade (Underkofler 1972). The active ingredients of these preparations have since been elucidated and isolated from a variety of sources.

In addition to papaya-derived papain, bromelain, from pineapple stem, and ficin, a fig tree sap extract, are the most well known plant-source enzymes applied to meat (Fawcett and McDowell 1987; Ockerman 1991), although numerous other novel sources have been tapped for enzymes with potential to affect positive changes in meat. Robbins and Cohen (1976) demonstrated the activity of catheptic enzymes from bovine spleen extracts. Wada et al. (2002) observed reductions in shear force with the use of actinidin, a thiol protease extracted from kiwi fruit. Stefanek et al. (2002) reported more consistent tenderization results using *Rhizomucor*-derived NCT, a fungal source enzyme, in comparison to bromelain and ficin. Ashie et al. (2002) tested an aspartic protease from *Aspergillys oryzae* and reported its effectiveness at low meat pH and in the presence of

0.4% phosphate. Furthermore, its activity was controllable by refrigeration and heat deactivation. Foegeding and Larick (1986) used a collagenase of bacterial origin that demonstrated greater specificity towards collagen and away from salt-soluble myofibrillar proteins than bromelain, ficin, and papain. Takagi et al. (1992) developed an alkaline elastase of bacterial origin with strong specificity for connective tissues. Other bacterial sources included cheese-making proteases (Cronlund and Woychik 1986) and thermophiles from Mount Erebus, Antarctica and Rotorua, New Zealand (Wilson et al. 1992). Fawcett and McDowell (1987) discussed that the development of enzymes from bacterial and fungal sources may be a move towards greater specificity and determinable temperature and pH levels for activity; however, these specifications would require an absolutely clear definition of the objective of application in order to achieve positive results.

Early attempts at enzyme application to meat included surface treatments (Weir et al. 1958) and antemortem vascular infusion (Underkofler 1972). Fogle et al. (1982) presented some of the first work on enzyme injection using a hand-held syringe-type injector. McKeith et al. (1994) described the incorporation of enzymes with the use of an automated injection system. This group reported that injection was the best method of enzyme introduction when compared to the inadequacy of tumbling and the inconsistent results with dipping. A unique, but likely unfeasible, approach was presented by Gerelt et al. (2000). This group promoted enzyme solution uptake during rehydration of meat previously subjected to osmotic dehydration using contact-dehydration sheets.

The issue of enzyme activity control has, perhaps, been the greatest limitation to widespread use of this tenderization method since over-tenderization resulting from indiscriminant breakdown of both connective tissue and myofibrillar proteins can have seriously detrimental effects on texture and flavour (Cronlund and Woychik 1986; Fawcett and McDowell 1987; McKeith et al. 1994; Ashie et al. 2002). Ockerman et al. (1993) attempted to limit papain activity in uncooked meat but found a potato extract was ineffective, and ascorbic acid, while effective, produced off flavours. Currently the only practical means of enzyme control is inactivation during cooking (Ashie et al. 2002). Despite a temperature optimum beginning at 65°C (Fogle et al. 1982; Ockerman 1991),

Ashie et al. (2002) observed that papain was not readily inactivated at cooking temperatures and still showed maximal activity after cooking to 75°C.

A number of researchers have attempted to provide a description of the ideal enzyme for use in meat tenderization. In general these definitions include the ability of an enzyme to display specificity of the target tissue, to be functional at the relatively low pH of meat, and to be thermally controlled by refrigeration and cooking depending on the application (Cronlund and Woychik 1987; Takagi et al. 1992; Wilson et al. 1992). The advice of Fogle et al. (1982) regarding the individual tailoring of enzyme levels to complement enzyme type and cooking methods is prudent since the environment in which an enzyme functions is critical to its catalytic activity. If targeted appropriately and used carefully to maintain control of activity, injected enzymes could be a feasible method to ameliorate toughness in otherwise low-valued muscles.

1.6. An Overview of the Chapters to Follow

Because of the precedent for its use in meat research, the *Longissimus thoracis et lumborum* was used as the model muscle for the mapping system. Once the method was established, a treatment known to improve instrumental tenderness in the *Longissimus* was applied to determine if the mapping system was useful for assessing changes resulting from the treatment, while simultaneously permitting examination of a related muscle characteristic. Furthermore, two additional treatments were applied to the *Longissimus* for its comparison to several other muscles and to determine an effective, muscle specific method of improving instrumental tenderness. The mapping method was then applied to the *Semitendinosus*, a muscle of interest due to its unusual characteristics and typical toughness, in order to study its inherent shear trends. Based on previous results, it appeared that a more rigorous tenderness enhancement approach was required for the *Semitendinosus*, and the final experiments investigated the effects of several injection treatments.

Table 1.1. Summary of highlights from a selection of available phosphate injection research using pork muscle.

Authors	Muscle	Injection characteristics	Primary injection results
Cannon et al. 1993	*Longissimus Semimembranosus*	5.0% P[z]; 2.5% NaCl; 1.5% dextrose; 1.5% vegamine at 10% injection[y]	Improved juiciness and less purge loss; Increased pH; Improved tenderness; Lowered shear
Sutton et al. 1997	*Longissimus*	0.2, 0.4% P; 1.0, 2.0% NaL[x] at 10% injection	Decreased purge loss; Increased pump yield; Improved juiciness; No effect on shear
Brewer et al. 1999	*Longissimus* Normal, PSE, DFD	0.3% P; 0.25% NaCl at 10% injection	Improved tenderness and juiciness; Amelioration of PSE texture
Sheard et al. 1999	*Longissimus*	3.0, 5.0% P at 5, 10% injection	Lowered cooking loss; Improved tenderness and juiciness; Increased abnormal flavour
Prestat et al. 2002	*Longissimus*	0.35% P; 0.35% NaCl; 3% NaL; 2.5% KL/KA[w] at 10% injection	Improved sensory attributes; Phosphate and NaL most effective flavour and texture enhancers
Brewer et al. 2002	*Longissimus*	0.4% P; 0.4% NaCl at 6, 12, 18 % injection	Consumers found injected samples appealing; texture and overall acceptability paralleled purchase intent

[z]Phosphate
[y]Percent over original weight
[x]Sodium lactate
[w]Combined potassium lactate and potassium acetate

Table 1.2. Summary of highlights from a selection of available phosphate injection research using beef muscle.

Authors	Muscle	Injection characteristics	Primary injection results
Vote et al. 2000	*Longissimus*	0.25% P[z]; 0.50% NaCl; 2.5% NaL[y] at 7.5, 10, 12.5, 15% injection[x]	Combined treatments improved tenderness, juiciness, flavour; Phosphate alone did not improve tenderness and imparted off-flavours
Boles & Shand 2001	*Semimembranosus* *Biceps femoris* *Semitendinosus* *Pectoralis profundus*	0.3% P; 1.8% NaCl; 1.0% sugar at 10, 25, 50% injection	Increased tenderness and decreased variability; Improved cooking yield; Increased pH
Robbins et al. 2002	*Longissimus*	0.4% P; 0.4% NaCl at 10% injection	Increased juiciness, tenderness, saltiness
McGee et al. 2003	*Semimembranosus*	0.25% P; 0.35% NaCl at 5, 7, 9% injection	Lowered shear and cooking loss; More favourable sensory attributes
Robbins et al. 2003	*Semimembranosus*	0.4% P; 0.4% NaCl at 10% injection	Lowered shear; Improved tenderness

[z]Phosphate
[y]Sodium lactate
[x]Percent over original weight

1.8. Literature Cited

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Mapping of Warner-Bratzler shear gradients in beef *Longissimus thoracis et lumborum*

2.1. Introduction

That tenderness is a critically important eating quality trait is undisputed. Lack of tenderness consistency has long been identified as a challenge to the meat industry on a worldwide basis (Sørheim and Hildrum 2002), and solving the problem of inconsistent beef quality has been identified as a top research priority in North America (Laycraft 1994; Koohmaraie et al. 1996). With 100% tenderness acceptability as the ultimate production goal for the retail beef market (Morgan et al. 1991; Beef Information Centre 2002), deeper knowledge of the inherent patterns of tenderness in beef muscles is required.

In a paper outlining standard procedures for meat quality measurements of beef according to the Commission of the European Communities Working Group on Meat Quality, Boccard et al. (1981) indicated that if economy dictated only one muscle could be used, the muscle of choice should be the *Longissimus*. The *Longissimus* muscle has been the traditional focus of commercial carcass assessment (Gariépy et al. 1990) and for estimation of the quantity of meat in a carcass (Henrickson and Mjoseth 1964). Furthermore, beef-industry funded research often focuses on the *Longissimus thoracis et lumborum* (LTL) since it is merchandised as a high-end steak cut (ribeye and striploin). Bailey (1972) indicated that the best quality meat, that which is suitable for grilling and roasting, is located in the proximal hind limb and the dorsal area posterior to the fifth rib. Since grilling is the primary preparation method for the LTL, and because this is an "unforgiving" method of cooking, the potential for consumer detection of quality flaws is accentuated.

As discussed in Chapter One, there are myriad papers reporting the tenderness variability in both the transverse and longitudinal planes of beef LTL (for example: Cover et al. 1962, Smith et al. 1969, Martin et al. 1970, Williams et al. 1983, Zuckerman et al. 2002, Kerth et al. 2002); however, these reports tend to be limited by the extent of

material tested. Conclusions about transverse tenderness gradients are often based on a single steak location. Results from an LTL segment have then been extrapolated to describe longitudinal tenderness trends in a muscle of considerable overall length (>1 m). In some cases, a lack of reporting on anatomical landmarks orienting sample collection within the carcass makes even casual comparison of results difficult. The divergence of results from these reports suggests that a limited sampling area hinders the development of a complete "tenderness map" of the LTL. It may also indicate that results in one portion of the muscle cannot be extrapolated to the entire muscle or other muscles within the carcass. While the recent shear mapping reports (Kerth et al. 2002; Zuckerman et al. 2002) have addressed some of these issues they also present certain limitations, as discussed in Chapter One.

The objectives of this experiment were: 1) to develop a technique to measure Warner-Bratzler shear force values of an entire muscle while maintaining three dimensional orientation and orientation to the skeleton; 2) to use the technique to conduct a thorough examination of tenderness gradients in beef *Longissimus thoracis et lumborum* and to identify possible causes for such trends; and 3) to suggest appropriate intervention strategies for tenderness enhancement based on analysis of shear trends.

2.2. Materials and Methods

2.2.1. Animal Processing

Animals used in this study were produced at the on-site beef production unit at the Agriculture and Agri-Food Canada Lacombe Research Centre (AAFC-LRC) in Lacombe, Alberta. All animals were British-type beef cattle finished on a grain/silage diet to 16-17 months of age and a mean liveweight of 582.2 kg ± 11.1 SEM. On the morning of each of six kill days, one heifer was transported ~2 km by truck from the beef unit to the research abattoir also located at AAFC-LRC. Animal delivery was directly followed by slaughter with no intervening lairage time. Once carcass dressing to commercial specifications was completed, all adipose, lean, and connective tissues overlying the *Longissimus thoracis et lumborum* were removed from both left and right sides. This served to expose the length of the LTL from the caudal end (anatomical reference terms

are summarized in Table 2.1) of the sixth lumbar vertebra to the cranial end of the fourth thoracic vertebra.

In order to moderate the carcass chilling conditions and reduce the potential for cold shortening in the exposed LTL, carcass chilling commenced in a cooler maintained at near 8.5°C (range 7.9-9.2°C). At 7.5 h postmortem, sides were moved to a cooler held at conventional chilling temperature (mean 0.8°C; range –0.4-2.0°C) for the remainder of the cooling period. For the duration of carcass chilling, internal temperature of the LTL was monitored and recorded. Thermocouple probes were inserted along the mid-line of the LTL at the approximate mid-point of muscle depth (2-3 cm varying with longitudinal location), at four locations corresponding to: the sixth lumbar, second lumbar, eleventh thoracic, and the fourth thoracic vertebrae. Thermocouple leads were connected to a junction box (Hewlett Packard 34970A Data Acquisition Switch Unit, Loveland, CO) and data were captured using HP Benchlink Data Logger software.

2.2.2. Muscle Removal and Division

At 24 h postmortem, thermocouple probes were removed, and marks placed along the length of the LTL to indicate reference points corresponding to lumbar and thoracic vertebrae prior to removal of the muscle from the carcass. Markings were made using food grade ink and were situated such that the line indicated the level of the caudal end of each corresponding vertebra. A second set of markings was then applied to indicate the mid-line and medial and lateral edges of the muscle. With the overlying tissues removed from the LTL, the medial and lateral edges were readily accessible and were determined to be the points furthest from the mid-line along the transition from the superficial to deep aspects of the muscle. The mid-line was the centre point between the two edges that varied in distance from one another (20-7 cm) along the length of the muscle as the LTL tapered towards the thoracic end. The muscles were then removed from both sides. In order to mark a common, cross-sectional centre point across all steaks, the junction of the mid-line, medial and lateral edges (Figure 2.1), previously indicated on the external surface of the whole muscle, was marked on the cranial face of the cross-sectional surface. The LTL was then cut into 4 cm steaks, beginning at the fifth lumbar marking and proceeding to the fourth thoracic marking, discarding sections destroyed by

temperature probes. In preparation for cooking, steaks were placed on a tray, with the cranial surface upwards, labeled, covered with plastic, and removed to the kitchen.

2.2.3. Cooking

Convection oven (Model X-80E, Bakers Pride, New Rochelle, NY) cooking was employed in order to maximize cooking uniformity while using a method that would not be unusual to beef consumers. With probes entering the medial side, thermocouples were placed in the geometric centre of each steak in order to continuously monitor internal temperature during cooking using a Hewlett Packard 34970A Data Acquisition Switch Unit and a computer equipped with HP Benchlink software. Steaks were placed, cranial face upwards, on wire cooking racks, to permit air circulation, in an oven preheated to 177°C and removed when internal steak temperature reached 70°C. Once removed from the oven, all steaks were placed in individual plastic bags and submerged in an ice bath to arrest cooking. When the steaks were cooled to 35 - 40°C, excess purge was poured from the bags and samples were arranged on trays for refrigeration (4°C) until shear testing the following day.

2.2.4. Sample Preparation, Colour Measurement, and Warner-Bratzler Shear Testing

With the cranial cross sectional surface facing upwards, all steaks were divided into serial, rectangular shear samples. Using a twin scalpel with blades set at a 15 mm width, the surface of each steak was scored in a grid pattern that originated at the pre-marked centre point; the 0,0 intersection of the X (steak width: medial to lateral) and Y (steak depth: superficial to deep) axes (Figure 2.1). Following the surface markings, each steak was cut into strips parallel to the Y axis. Further division followed the grain of the muscle fibres now exposed. All tissue within the epimysium and which yielded a complete 15 x 15 mm sample was used for shear analysis. From the first four muscles, notations were made regarding samples with particularly high concentrations of fat and/or connective tissue. Prepared samples were arranged on a tray, lined with a laminated grid surface (Figure 2.2), in order to maintain locational orientation.

From a freshly exposed surface of each shear sample, an instrumental colour measurement was recorded using a Minolta CR-300 with Spectra QC-300 software (Minolta Canada Inc., Mississauga, ON) with light source C and a 2° observer angle. These data were collected as a non-destructive indicator of cooking uniformity. Samples were sheared on an Instron 4301 Materials Testing System (Burlington, ON) equipped with a Warner-Bratzler shear force cell and Series 9 software, and cross-head speed set to 200 $mm \cdot min^{-1}$. Peak load was recorded in kg.

2.2.5. Carcass Dissection

On a separate occasion, one beef steer was processed at the Lacombe Research Centre abattoir. The right carcass side was lowered from the rail system onto a table so as to permit access to the musculature in a position relatively similar to normal physiological posture. With a scalpel and knife, the tissue layers overlying the *Longissimus thoracis et lumborum* were identified and removed to expose the full length of the muscle. Cross sections were removed from the lumbar, central, and thoracic regions to permit closer examination of fibre direction. Notes, diagrams, and digital images were collected regarding LTL location, points of skeletal attachment, and muscle fibre direction within the LTL.

2.2.6. Data Preparation and Statistical Analyses

In order to maintain proper spatial orientation, data from the right side muscles were mirrored such that medial and lateral locations between sides were aligned before further analysis. Exploratory analysis (general linear model (GLM) procedure, SAS/STAT 1990) of location factors across the entire set of shear data indicated no significant difference between left and right sides. As such, left and right sides data were merged by shear sample location prior to further data analysis.

The entire set of shear data was subject to the univariate procedure of SAS (SAS 1985) in order to test the normality of the distribution. The Kolmogorov-Smirnov statistic, used where $N>2000$, indicated a non-normal distribution ($P<0.01$), with skewness and kurtosis values 0.74 and 0.61, respectively. When the data were plotted in a histogram (Figure 2.3), however, it was apparent that the core of the distribution had a

distinctly normal shape with a tail extending into the range of higher shear values. Logarithmic transformation of the data set reduced the skewness value, however, the distribution remained significantly non-normal. Since the distribution of the original data set was not sharply skewed in either direction and since analysis of variance is a procedure sufficiently robust to accommodate some deviation from its underlying assumptions (Steel and Torrie 1980), the original data set was used for all further analyses.

Prior to analysis, CIE L*, a*, and b* (Commision Internationale de l'Éclairage 1978) values were converted to colour (hue = arctan[b*/a*]) and colour saturation (chroma = $[a^{*2}+b^{*2}]^{0.5}$). L*, chroma, and hue were evaluated, using the GLM procedure, as potential covariates in the analysis of shear values. Only hue was significant as a covariate, however, the addition of this variable to the analysis did not change the pattern of significance/non-significance of the location effects. Using the regression procedure of SAS (SAS/STAT 1990), further examination of the relationship between hue and shear indicated a significant R^2 value (0.0230, $P<0.0001$), however, this showed that only 2.3% of the variation in shear could be attributed to hue. Considering the much greater known effects of location, the contribution of hue to patterns of shear values was determined to be minimal and was not considered in further analysis.

To permit closer examination of longitudinal sections of the LTL, data from the entire length were divided into three "blocks". Analysis of shear data with the GLM procedure included animal, steak, and shear sample in the model. Means separation with the probability of difference option was used to determine the break points for block creation. Longitudinal block groupings and their alignment to vertebral locations are presented in Figure 2.4, which indicates block 1(lumbar) = fifth lumbar (L5) to the second/first lumbar (L2/1), block 2 (central) = first lumbar/thirteen thoracic (L1/T13) to T10/9, and block 3 (thoracic) = T9 to T5.

Because of the nature of LTL muscle shape, with variation in cross sectional area along its length (Figure 2.5), "zones" were established for the analysis of superficial-deep and medial-lateral trends, rather than using individual shear sample locations. Three superficial-deep zones were established within each block (Figure 2.6a), while the number of medial-lateral zones varied from five to ten according to the width of steaks in

each block (Figure 2.6b). Within each block, analysis of zones, using the GLM procedure included animal, steak, and zone and their interactions. Effects were considered significant at the 0.05 level and means separations were completed using the probability of difference option.

Standard deviation data were also analysed using the GLM procedure. Standard deviations were calculated across animal and side for each shear sample location. Analysis of standard deviation at the higher levels of spatial organization, for example longitudinal block, was then based on a population of standard deviations, those from the smaller organizational sub-units, with its own variance, thus permitting the use of the GLM procedure for the statistical evaluation of variability.

Correlation analysis (SAS 1985) of various locations within the LTL was conducted to assess the strength of the relationship of Warner-Bratzler shear values amongst: longitudinal blocks, steaks within longitudinal blocks, selected medial-lateral zones across all longitudinal locations and within longitudinal blocks. Multiple comparison regression analysis was conducted using the regression procedure with the MaxR and Stepwise functions for the prediction of average shear force by various sample locations within longitudinal blocks. MaxR permitted the determination of the best one variable model, the best two variable model, and so on, for the maximization of R^2. Stepwise performed a similar function, while maintaining a check on the significance (maximum 0.15) of the variables added to the model.

Because of variability in shear sample make up (fat, lean, CT) further GLM analysis based on primary tissue type was conducted to examine the impact of tissue type on shear values.

2.3. Results and Discussion

2.3.1. Longitudinal Trends

Along the longitudinal axis of the LTL, shear was greatest in the central block (L1/T13 to T10/9, including the Canadian beef carcass grading site, T12/13), lowest in the thoracic block (T9 to T5), and intermediate in the lumbar block (L5 to L2/1) (Table 2.2). The range of shear values across steaks within the longitudinal blocks is demonstrated in Figure 2.7. Henrickson and Mjoseth (1964) reported very similar results

demonstrating that the *Longissimus* was toughest near T13, became more tender towards T7, and was intermediate in tenderness between L6 and L2.

In an examination of the posterior half of the *Longissimus*, Martin et al. (1970) reported contrasting data indicating lower shears near T11/12 with toughness increasing towards the lumbar region. Gariépy et al. (1990) also reported the lowest shears in the central (T12 to T8) versus thoracic (T7-T4) and lumbar (L5-T13) regions. Sampling in that study was limited, however, to the examination of one steak at each longitudinal location from each of which only three cores were sheared. Yet other reports have indicated no effect of longitudinal location (Williams et al. 1983), however, extremely limited sampling (Wheeler er al. 1996) and the use of taste panel tenderness evaluation (Jeremiah and Murray 1984) hindered comparison with the present results.

Variability within longitudinal blocks (Table 2.2) was greatest in the lumbar region, and decreased significantly toward the thoracic end of the muscle. Wheeler et al. (1996) also reported a lower shear standard deviation in this region of the LTL. Henrickson and Mjoseth (1964), however, indicated the greatest uniformity in shear was found in the lumbar location and recommended the use of that portion of the muscle for meat quality investigation. Longitudinal variation in shear values may be related to variability in the degree of contraction experienced during rigor mortis development at different locations along the length of the LTL. Unpublished time-lapse video data from our lab indicated a differential rigor contraction pattern along the length of the LTL during conventional chilling. Gariépy et al. (1990) also suggested differences in muscle restraint due to shackling during carcass dressing, carcass cooling, muscle fibre angle, metabolic fibre type, and connective tissue content as possible causes of intramuscular variation.

2.3.2. Cross Sectional Trends

2.3.2.1. *Superficial-deep direction*

Prior to carcass chilling, tissues overlying the LTL were removed to eliminate any extraneous influence on tenderness of varying depths of insulating tissues either along the length of the muscle or between muscles of different animals. Muscle depth along the length of the LTL varied from 3-4 cm at the extremities to 5-6 cm at the central locations.

A greater initial rate of temperature decline was observed in the thoracic region while temperature in the central area declined more gradually (Figure 2.8); however, the recorded temperatures in all locations indicated that the rule of thumb for avoidance of cold shortening, that is the maintenance of an internal muscle temperature above 10°C within the first 10 h postmortem (James and James 2002), was violated. If cold temperature exposure was the only factor in toughness/tenderness development, one would expect higher shear values at the superficial location while the deep muscle location would have lower shear values since cold shortening (Locker and Hagyard 1963; Marsh and Leet 1966) would be limited with an increased insulative effect of greater muscle thickness. While this statistically significant trend prevailed in the superficial to deep cross section of the lumbar region (Figure 2.9), the difference in mean shear value between the superficial and deep locations was less than 1 kg; that is, below the threshold for consumer detection (Aalhus et al. 1999). In the central and thoracic blocks, the trend was reversed with a significant gradient of increasing shear value from the superficial to deep locations indicating that the expected effect of temperature on shear value did not occur.

The trend in variability of shear values (Figure 2.10) in the superficial-deep dimension mirrored mean values with larger shear values having significantly greater variability, as indicated by standard deviation. This effect was similar to that reported by Dugan and Aalhus (1998) who indicated a strong positive correlation between the two factors. These authors also proposed several possible explanations for tenderness variability within a single beef steak, including protein degradation, ion concentration, contents of fat, collagen, and water, and variation in degree of rigor contraction. Although the occurrence of cold shortening was likely, some of the superficial/deep trends do not seem to be consistent with cold shortening being the sole factor influencing shear values, hence suggesting that tension during rigor development has a greater impact on cross sectional shear gradient than temperature during carcass cooling. This is particularly evident upon examination of the medial to lateral dimension.

2.3.2.2. Medial-lateral direction

In the medial to lateral cross section, shear gradients were significant and unique to each longitudinal segment (Figure 2.11). In the lumbar region there were significantly larger shear values on the medial side. With a move toward the lateral edge, the shear values declined along with the degree of difference between the zone locations. In the central portion of the LTL, the shear gradient was somewhat sigmoidal with multiple peaks and valleys traversing the cross section. Although Zuckerman et al. (2002) provided no definition of the longitudinal location within the *Longissimus*, the surface plot they published demonstrated a trend very similar in its serpentine pattern to the present results. Also in the current results, shear values in the thoracic area displayed a significant increase in shears from the medial to the lateral positions. No significant difference in standard deviation was observed in the central and thoracic blocks of the LTL; however, the lumbar region displayed significant variability with the gradient declining from medial to lateral (Figure 2.12). In general, the medial to lateral gradient was more pronounced than that observed in the superficial to deep cross section (Figure 2.13 a,b,c), an observation also reported by Kerth et al. (2002).

Many published reports indicate the presence of a tenderness gradient in the medial to lateral dimension of the *Longissimus*, but there is considerable discrepancy about the direction of this trend. Supporting a decreasing gradient from lateral to medial are reports by McBee and Wiles (1967), Hedrick et al. (1968), Smith et al. (1969), Martin et al. (1970), Williams et al. (1983), Berry (1993), and Kerth et al. (2002). Conversely, Cover et al. (1962), Hostetler and Ritchey (1964), Alsmeyer et al. (1965), and Crouse et al. (1989) reported a trend with larger shear values near the medial edge. Common amongst these reports is a limited amount of material subjected to testing and/or a lack of definition about the sampling location within the muscle. Quite evidently, shear variability is affected by sampling location, as well as sampling technique including carcass chilling, product ageing, cooking method, and core removal technique (Janz and Aalhus 2002; Jeremiah 2002). All of these factors play a role in the disparity of conclusions reported in the literature.

2.3.3. Internal Tension Development

Tension inherent to the LTL during establishment of rigor mortis appears to be a crucial influence on the development of the observed gradients and variability of shear values. The LTL has a complex pattern of skeletal attachment with no single points of origin and insertion. Along its length, this muscle contains multiple locations of skeletal connection with myofibres varying in angle relative to the longitudinal axis of the muscle. The points of origin of the *Longissimus* include the tubera coxae, crest, and ventral surface of the ilium, the sacral, lumbar, and thoracic spines, and the supraspinous ligament. Muscle fibres terminate, or insert, at the lumbar transverse and articular processes, the thoracic transverse processes, the spinous and transverse processes of the four posterior cervical vertebrae, and the lateral surfaces of the costal bones (Eisenhut et al. 1965; Jones et al. 2001). The heavy, fibrous connective tissue layer, or epimysium, covering the *Longissimus* muscle is tightly entwined with the points of origin (Eisenhut et al. 1965). The biomechanical function of the *Longissimus thoracis et lumborum* is extension and lateral flexion of the spine (Jones et al. 2001). Toughness is greatly influenced by the nature of the muscle attachment to the skeleton and the extent of stretch placed on the muscles during carcass suspension (McCrae et al. 1971). Any movement in the structures of *Longissimus* origin would cause a shift in muscle position, hence fibre angle and sarcomere length, particularly in the posterior portion of the muscle (Eisenhut et al. 1965).

Shear values were lowest and most consistent in the thoracic region of the LTL, indicating that tension development may be fairly uniform across this location. Carcass dissection observations indicated the oblique fibre direction of thoracic myofibres is uniform across the muscle, as compared to the central and lumbar areas in which fibre angle varies in the cross section. The smaller thoracic cross section may also promote more consistent development of rigor tension as compared to areas with a considerably wider cross section.

Focusing on the lumbar segment of the LTL, both shear values and standard deviation were significantly lower at the lateral versus medial side of the muscle. Berry (1993) reported the presence of unspecified, inherent properties in the lateral portion of the loin steaks that made it unlike other sections in terms of tenderness. Examination of

myofibre direction in the medial to lateral cross section of the lumbar region revealed a shift in fibre angle across the LTL (Figure 2.14). On the medial side fibres angled from caudal/medial to cranial/lateral. On the lateral side fibres were nearly parallel with the longitudinal axis of the muscle. Lower shear values and reduced variability could be explained by the differential tension placed on the myofibres during carcass suspension. In the medial area of the lumbar region strain is placed on the myofibres at an angle to their long axis. The lateral fibres experience force along their length possibly creating greater resistance to rigor contraction, along with longer sarcomere length, hence resulting in the lower shear values observed in the lateral versus medial region of the lumbar cross section. Furthermore, where the risk of cold shortening exists, the additional strain placed on the contractile mechanism in the lateral region may provide additional protection against the tendency for extreme contraction. Measurement of sarcomere lengths across the muscle and under different chilling conditions would be required to confirm this hypothesis.

Both skeletal attachment and intramuscular fibre arrangement affect muscle tension postmortem and have been implicated in controlling sarcomere length and associated characteristics (Locker 1960; Eisenhut et al. 1965). According to Koohmaraie et al. (1996), meat toughening is due to sarcomere shortening during rigor development and in the absence of sarcomere shortening shear force does not increase. During conventional carcass suspension from the Achilles tendon, tension on the *Longissimus*, particularly in the posterior half of the muscle, is permitted to slacken. Extension of the hip joint, hence flexion of the spine, results in decreased strain placed on the LTL, a muscle designed for spinal extension. Reduced resistance to ongoing postmortem muscle contraction increases the potential for extensive sarcomere shortening to develop as a result of rigor tension. Even muscles fixed in overall length by firm attachment to the skeletal framework are capable of localized shortening (Marsh and Leet 1966). The resulting areas of contracted and stretched tissue contribute directly to tenderness variability. As early as 1960, Locker suggested that *Longissimus* quality would probably be improved by hanging the carcass such that the LTL is stretched and prevented from shortening. Since that time various methods of altered suspension and skeletal alteration have been reported (Hostetler et al. (1970); Smith et al. (1971); Bouton and Harris

(1972); Wang et al. (1994); Ludwig et al. (1997); Aalhus et al. (1999)) and are ideal for increasing tension, hence tenderness, in the LTL.

2.3.4. Correlation and Regression Analyses

Correlation coefficients across various anatomical locations are reported in Appendix A. Amongst longitudinal locations, only central and thoracic regions were at all correlated and the relationship was very weak ($R = -0.08$; $P = 0.04$). When steaks within the longitudinal regions were examined, only a small number of seemingly random relationships were observed. The thoracic segment had the greatest number of significant correlations, 6 out of 13 possible comparisons. This segment was also the most tender and had the greatest uniformity in instrumental tenderness (lowest standard deviation) as compared to the central and lumbar regions. The LTL was further subdivided as comparisons were made longitudinally along medial, central, and lateral vectors. Again, there was a sparse occurrence of significant relationships between locations, with no trend on which to base solid predictions of shear force. As previously discussed, fluctuations in Warner-Bratzler shear force were observed in all dimensions of the LTL, so the lack of a large number of strong relationships between individual locations was not surprising. As such, the analysis was not expanded to regression since the relationships would bear little predictive power.

Multiple variable linear regression analysis, however, was conducted to evaluate the predictive ability of shear sample locations for the average shear values and to determine the combinations of locations required to maximize this predictive value, as indicated by R^2. A separate analysis was conducted for each longitudinal segment since the total number of shear samples varied with the general shape of the steaks from each region. In all regions, the best single location for average shear force prediction was near the centre of the steaks (refer to Figure 2.6a for sample numbers), an observation also reported by Kerth et al. (2002). With the inclusion of only three to four samples, $R^2>0.90$ was achieved (Tables 2.3, 2.4, and 2.5).

2.3.5. Tissue Type

In the interest of characterizing the entire LTL muscle, all material within the epimysium that constituted a complete sample (15 x 15 mm) was sheared. As such, some samples contained large concentrations of connective and adipose tissues. In comparison to the range in shear values experienced amongst different locations, the difference in shear values between tissue, although significant (Table 2.6), was relatively small, suggesting a minimal contribution to shear variability. Samples composed mainly of fatty tissue had the lowest shear value indicating that there may be a biological basis for increased tenderness with increased marbling. Samples containing a mixture of fat and connective tissue generally contained a portion of blood vessel, a tissue high in elastin, and yielded the greatest shear value of the various tissue types. Interestingly, samples of primarily collagenous connective tissue had shear values virtually identical to those containing mostly lean muscle tissue. While collagen possesses a high tensile strength, its shear strength is minimal. This indicates that attempting to avoid areas of obvious connective tissue may only serve to reduce the available sampling area since regions of primarily collagenous connective tissue did not have an increased shear value over that of lean tissue. These are preliminary data, however, and further compositional analysis of shear samples would be necessary to verify the results.

2.4. Implications

The present procedure included extensive sampling volume using a method that is easily repeated due to the provision of specific anatomical landmarks. Furthermore, all sampling was conducted relative to a common centre point and parallel to muscle fibre direction to aid with the reproducibility of the technique. By including all material within the epimysial layer, the sampling area was maximized while varying tissue types did not compromise shear data. Ageing, although a simple and effective means of enhancing tenderness, was not included in the treatment so as not to introduce time as a fourth dimension.

Although an attempt was made to moderate cooler temperature during the initial chilling period, the duration of this period was not sufficient to maintain internal muscle temperature outside the cold shortening risk zone. As such, the occurrence of cold

shortening was very likely, although in the absence of sarcomere length data, this cannot be confirmed. Since the tissue overlying the LTL was removed, cold shortening was a risk along the entire length of the muscle, rather than in localized regions. As such, the observed gradients in shear values were likely exaggerated, rather than altered, as compared to a situation where cold shortening was not a risk. The comparison of absolute shear values observed in the present study to those from similar studies is then limited by this complicating factor.

Gradients in shear values and shear variability were observed in all planes within the LTL, an inherent characteristic that must be given consideration when conducting tenderness evaluation. Both Sharrah et al. (1965) and Reuter et al. (2002) indicated that variablity within, rather than between, muscles may be of greater importance. Segars et al. (1974) furthered the argument, stating that there is as much or more variation in tenderness across a muscle as along the length. Dugan and Aalhus (1998) concluded that shear differences between shear cores from the same steak can be sufficiently large that apparent outliers might be regarded as artifactual. Since shear sample location can be a confounding factor in tenderness evaluation, demonstrated by the lack of strong and consistent correlations between locations, sampling position must be held constant when conducting shear force testing across numerous steaks. Wheeler et al. (1996) reported that repeatability maximized by shearing 10 cores per steak, although after the fifth core, little improvement in repeatability was realized. Cover et al. (1962) suggested that averaging shears from four wide spread locations could overcome the lack of tenderness homogeneity across a steak. The present results support this statement; however, where material or time limitations dictate that only a few select measures can be taken, one must cautiously consider that potentially valuable information can be lost with averaging (Segars et al. 1974) and small areas of tough shears may be diluted or discarded as outliers (Zuckerman et al. 2002).

Much shear variability has been observed within the *Longissimus thoracis et lumborum*, and its oblique fibre direction renders it atypical of the general musculature (McCrae et al. 1971). As such, one cannot expect results from the LTL to be representative of any other muscle in the carcass, an argument also presented by Cover et al. (1962) and Sharrah et al. (1965). The LTL, even localized segments within the LTL,

display unique patterns of postmortem contraction resulting in toughness/tenderness gradients in all dimensions. The current results indicate that results from a single location cannot be extrapolated to the entire length of the muscle let alone to other muscles. The LTL may be valuable as a benchmark since it is often the focus of meat quality studies, but it is invalid to use it as an indicator for the entire carcass.

It is clear that the degree of contraction in which muscles enter rigor mortis is a significant factor in determining tenderness (Locker 1960). The final state of the muscle is determined, in large part, by the tension imposed on it by carcass suspension. *Longissimus thoracis et lumborum* quality would be improved by hanging the carcass such that the LTL is stretched and prevented from shortening or by sufficiently controlling the onset of rigor mortis by cooler temperature regulation.

2.5. Tables

Table 2.1. Anatomical reference terms

Anatomical term	Definition
Cranial	A directional term referring to a location nearer the head[y]
Caudal	A directional term referring to a location nearer the tail[y]
Median plane	The craniocaudal plane dividing the body into left and right halves[y]
Transverse plane	A plane perpendicular to the median plane dividing the body into cranial and caudal segments[y]
Longitudinal plane/axis[z]	Used with similar meaning to median, but specific to the Longissimus muscle
Medial	A structure nearer than another to the median plane[x]
Lateral	A structure farther than another from the median plane[x]
Superficial	A structure nearer than another to the exterior surface of the body[y]
Deep	A structure nearer than another to the interior or centre of gravity of the body[y]
Central[z]	The mid-point between superficial and deep, medial and lateral, or lumbar and thoracic, depending on the application

[z]Non-scientific term
[y]Frandson and Spurgeon (1992)
[x]Getty (1975)

Table 2.2. Least squares means and standard deviation of shear values in longitudinal locations in the *Longissimus thoracis et lumborum*

Longitudinal location	Shear (kg)	SEM	Standard deviation	SEM
Lumbar	11.46a	0.08	2.44a	0.77
Central	11.77b	0.06	2.12b	0.76
Thoracic	10.79c	0.08	1.72c	0.10

a,b,c Values in the same column followed by different letters are significantly different ($P<0.05$)

Table 2.3. Regression co-efficients from multiple regression using MaxR and Stepwise selection for the prediction of average Warner-Bratzler shear force from individual shear sample locations within the lumbar region.

MaxR selection			Stepwise selection	
Number of variables in best model	Shear sample location	R^2	Shear sample location	P
1	15	0.7471	15	<0.01
2	15,13	0.8717	13	<0.01
3	15,13,12	0.9222	12	<0.01
4	15,13,12,25	0.9555	25	<0.01
5	15,13,12,25,2	0.9762	2	<0.01
6	15,13,12,25,2,9	0.9821	9	0.08
7	15,13,12,25,2,9,20	0.9877	20	0.06
8	15,13,12,25,2,9,20,27	0.9912	27	0.09
9	15,13,12,25,2,9,20,27,17	0.9953	17	0.03
10	15,13,12,25,2,9,20,27,17,7	0.9975	7	0.05
11	15,13,12,25,2,9,20,27,17,7,4	0.9989	4	0.03
12	15,13,12,25,2,9,20,27,17,7,4,3[z]	0.9995	3	0.07

[z]Variable addition stopped at the limits significance indicated by stepwise selection

Table 2.4. Regression co-efficients from multiple regression using MaxR and Stepwise selection for the prediction of average Warner-Bratzler shear force from individual shear sample locations within the central region.

	MaxR selection		Stepwise selection	
Number of variables in best model	Shear sample location	R^2	Shear sample location	P
1	26	0.7617	26	<0.01
2	26,23	0.8923	23	<0.01
	26,23,13		13	<0.01
3	26,-23,13,+24[z]	0.9270		
4	26,13,24,17	0.9469	17	<0.01
5	26,13,24,17,5	0.9627	5	<0.01
6	26,13,24,17,5,23	0.9712	24	<0.01
7	26,13,24,17,5,23,6	0.9806	6	<0.01
	26,13,24,17,5,23,6,18	0.9831	18	0.08
8	26,13,24,17,5,23,-6,18,+4	0.9841	27	0.02
9	26,13,24,17,5,23,18,4,27	0.9911	4	<0.01
10	26,13,24,17,5,23,18,4,27,12	0.9929	-6	0.55
11	26,13,24,17,5,23,18,4,27,12,22	0.9940	12	0.03
12	26,13,24,17,5,23,18,4,27,12,22,16	0.9951	22	0.07
13	26,13,24,17,5,23,18,4,27,12,22,16,21	0.9959	16	0.06
14	26,13,24,17,5,23,18,4,27,12,22,16,21,8	0.9965	21	0.08
15	26,13,24,17,5,23,18,4,27,12,22,16,21,8,6	0.9972	8	0.11
	26,13,24,17,5,23,18,4,27,12,22,16,21,8,6,14	0.9977	+6	0.07
16	26,13,24,17,5,23,18,4,27,12,22,16,21,8,6,-14,+19	0.9978	14	0.09
17	26,13,24,17,5,23,18,4,27,12,22,16,21,8,6,19,18	0.9983	19	0.04
18	26,13,24,17,5,23,18,4,27,12,22,16,21,8,6,19,18,25[y]	0.9986	25	0.13

[z]In steps requiring multiple model changes, - and + signs indicate removal or addition of a variable from the model, respectively

[y]Variable addition stopped at the limits significance indicated by stepwise selection

Table 2.5. Regression co-efficients from multiple regression using MaxR and Stepwise selection for the prediction of average Warner-Bratzler shear force from individual shear sample locations within the thoracic region.

MaxR selection			Stepwise selection	
Number of variables in best model	Shear sample location	R^2	Shear sample location	P
1	16	0.6533	16	<0.01
2	16,24	0.8126	24	<0.01
3	16,24,18	0.8836	18	<0.01
	16,24,18,23	0.9225	23	0.01
	16,24,-18,23,+17[z]	0.9256		
	16,-24,23,17,+25	0.9270	25	0.03
	-16,23,17,25,+27	0.9464	27	<0.01
4	-23,17,25,27,+18	0.9609	17	<0.01
5	23,17,25,27,18	0.9766		
6	23,17,25,27,18,16	0.9825		
7	23,17,25,27,18,16,24	0.9907		
8	23,17,25,27,18,16,24,14	0.9955	14	<0.01
9	23,17,25,27,18,16,24,14,15	0.9967	15	0.07
	23,17,25,27,18,16,24,14,15,5[y]	0.9977	5	0.07

[z]In steps requiring multiple model changes, - and + signs indicate removal or addition of a variable from the model, respectively

[y]Variable addition stopped at the limits significance indicated by stepwise selection

Table 2.6. Least squares means of shear values for Warner-Bratzler shear samples of varying predominant tissue types.

Predominant tissue type	Shear (kg)
Lean	11.98b
Fat	10.89a
CT[z]	11.96bc
Fat/CT	13.03c

[z]Connective tissue

a,b,c Values followed by different letters are significantly different (P<0.05)

2.6. Figures

Figure 2.1. Indication of orientation of each steak for rectangular shear sample preparation around a common origin (0,0) relative to X and Y axes.

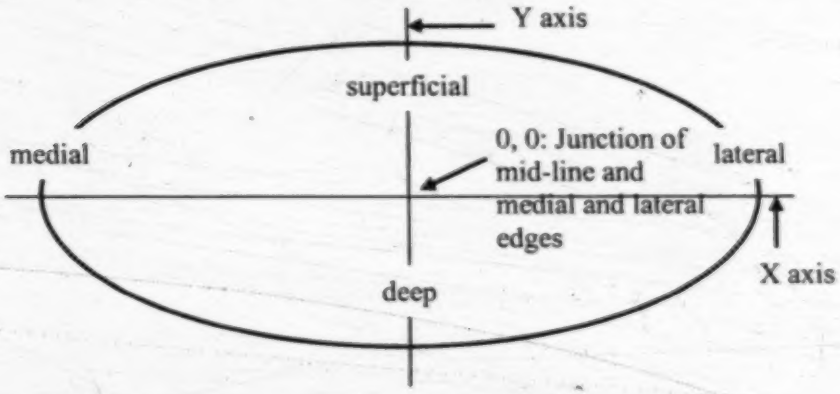


Figure 2.2. Twin blade scalpel for shear sample preparation (**a**); steak cut into strips parallel to the Y axis (**b**); sample preparation following myofibre grain (**c**); prepared samples on grid (**d**).

Figure 2.3. Distribution of all shear values observed in the *Longissimus thoracis et lumborum*.

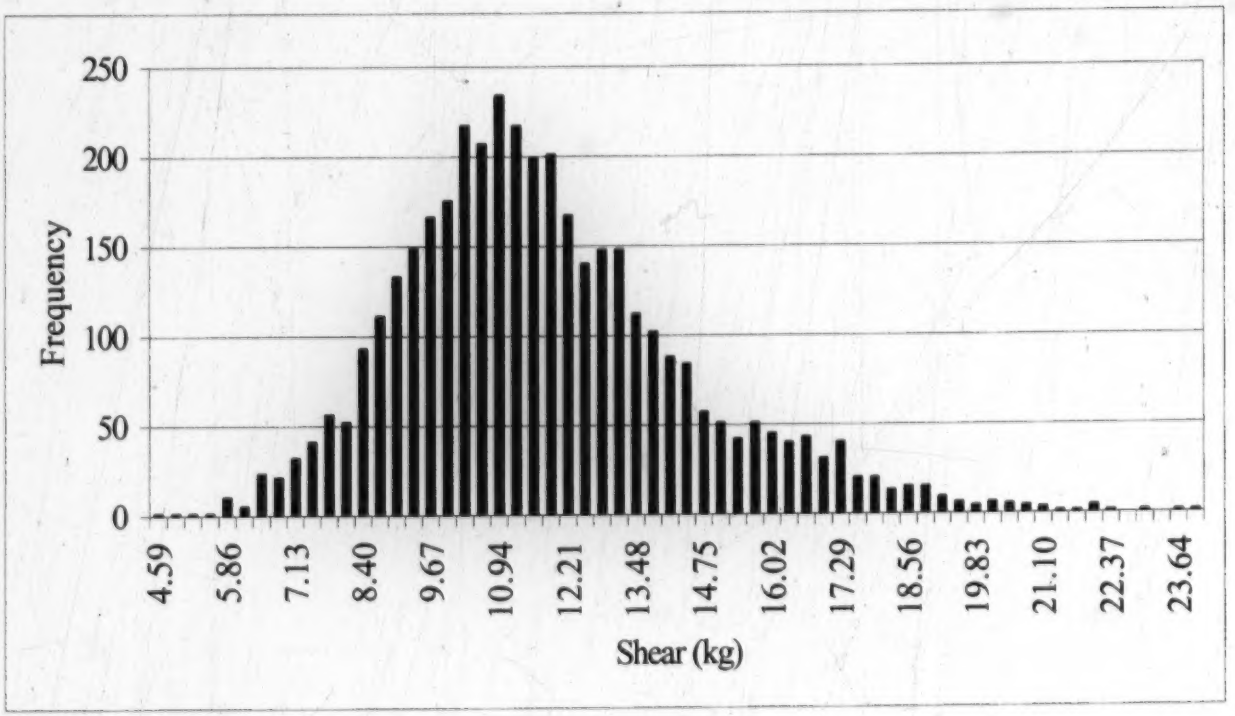
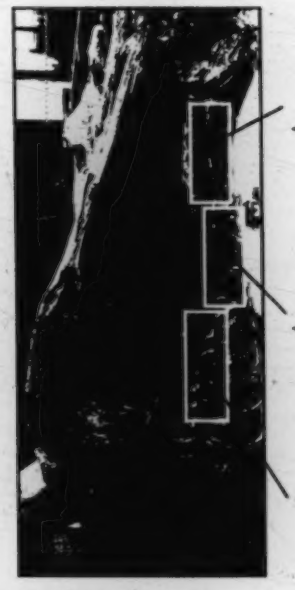


Figure 2.4. Orientation of steaks and longitudinal block groupings with vertebral locations.



Vertebra[z]	Steak number	Longitudinal block[x]
L5	1	1
L5/4[y]	2	1
L4/3	3	1
L3	4	1
L2[w]		
L2/1	5	1
L1/T13	6	2
T13	7	2
T12	8	2
T11[w]		
T10	9	2
T10/9	10	2
T9	11	3
T8	12	3
T7	13	3
T6	14	3
T5	15	3

[z]L=lumbar; T=thoracic
[y]Since vertebrae were wider than the 4 cm steaks, some overlap of vertebrae exists
[x]For further analysis, steaks were groups together as longitudinal blocks
[w]Destroyed during measurement of temperature

Figure 2.5. Demonstration of the varying cross sectional shape of the *Longissimus thoracis et lumborum* in the lumbar (**a**), central (**b**), and thoracic (**c**) regions (adapted from Jones et al. 2001)

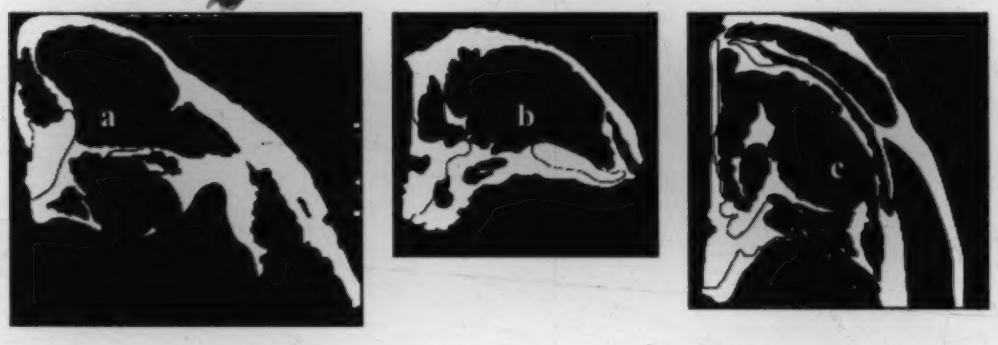
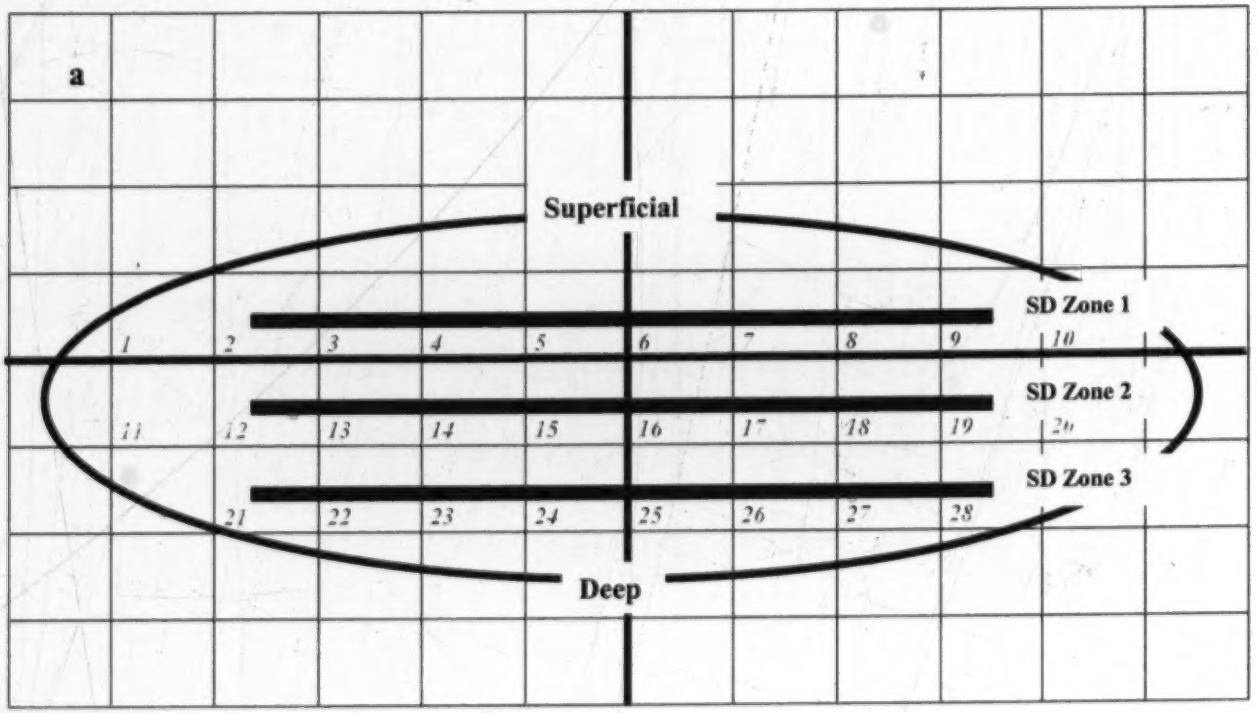


Figure 2.6. Schematic representation of superficial-deep (**a**) and medial-lateral (**b**) zones assigned for cross sectional shear gradient analysis in the *Longissimus thoracis et lumborum*. Ovals represents a steak superimposed over a grid system oriented around a central point of origin. Each coordinate labeled square within the oval represents a sample for Warner-Bratzler shear testing. Bold horizontal lines indicate samples included in SD zones and bold vertical lines indicate samples included in ML zones. Since steak size, hence the number of possible shear samples, varied along muscle length, the number of ML zones varied from 5-10 amongst longitudinal block locations. Italicized numbers 1 to 27 indicate individual shear sample locations.



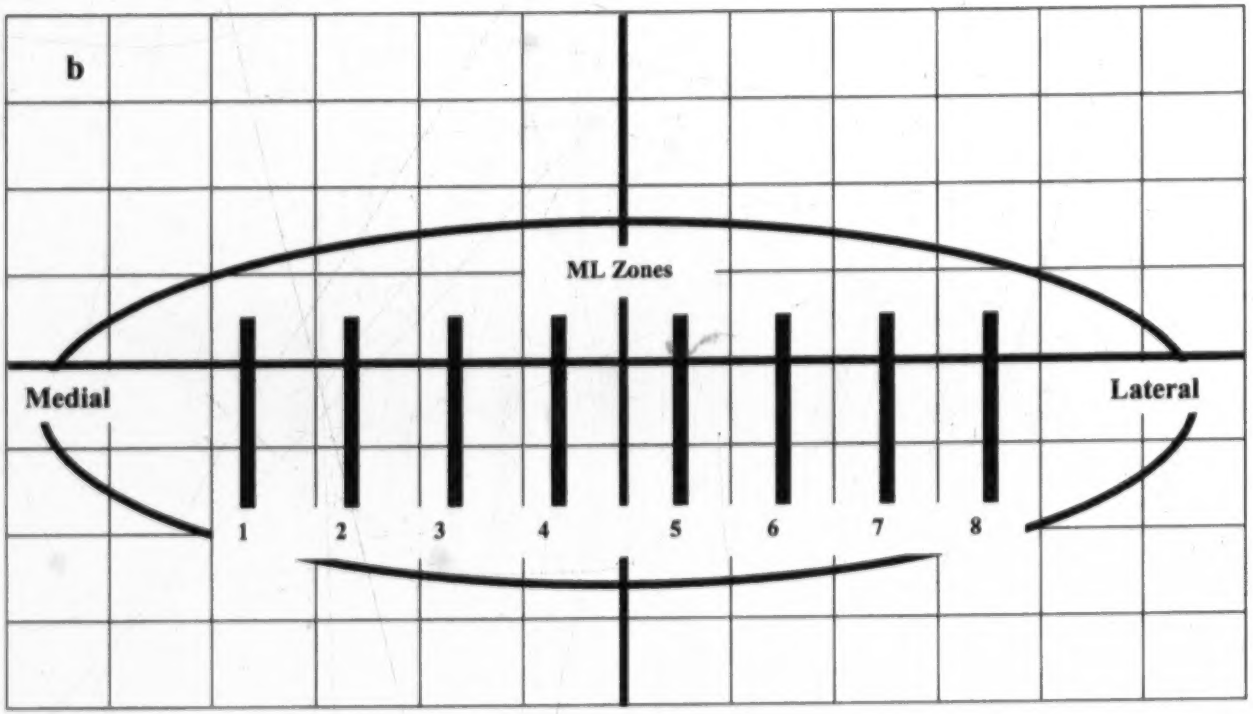
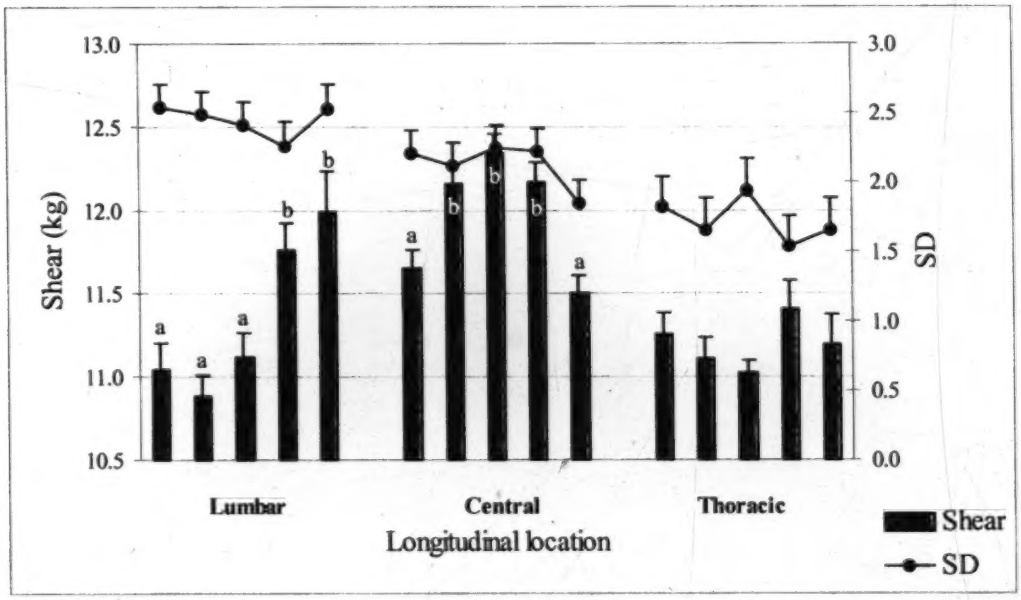

b
ML Zones
Medial
Lateral
1
2
3
4
5
6
7
8

Figure 2.7. Gradient of mean Warner-Bratzler shear values and standard deviation (SD) of steaks within longitudinal locations in the *Longissimus thoracis et lumborum*. Vertical bars are standard errors.



a,b Shear value bars within longitudinal location groups marked with different letters are significantly different ($P<0.05$)

Figure 2.8. Cooler temperature and temperature decline at several vertebral locations along the *Longissimus thoracis et lumborum* during carcass cooling.

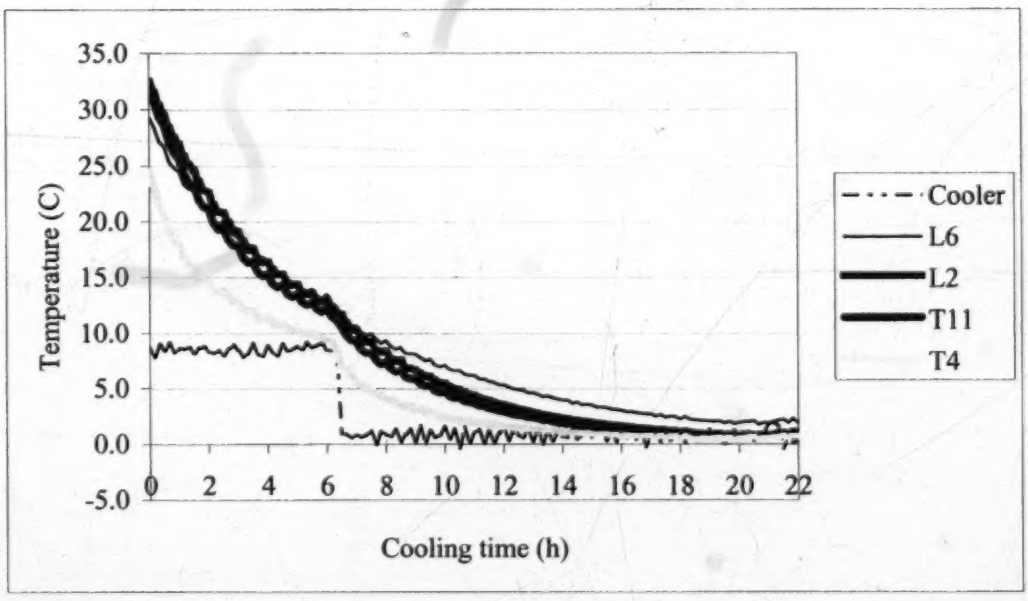
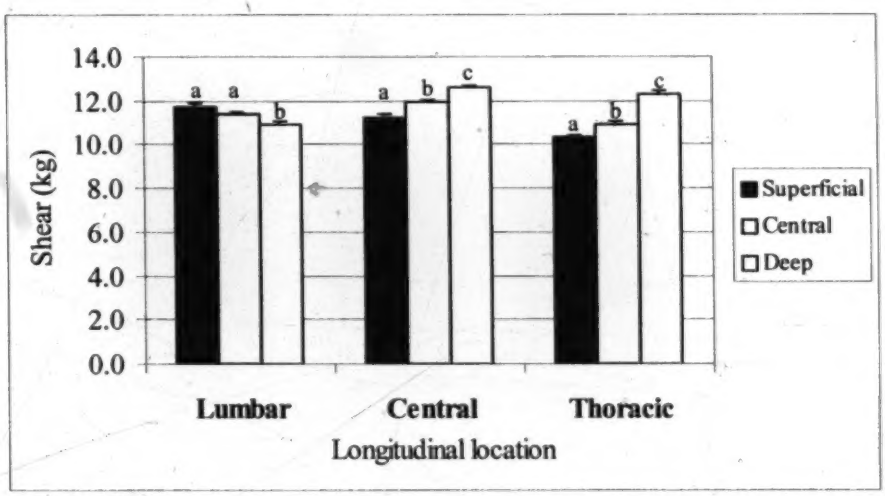
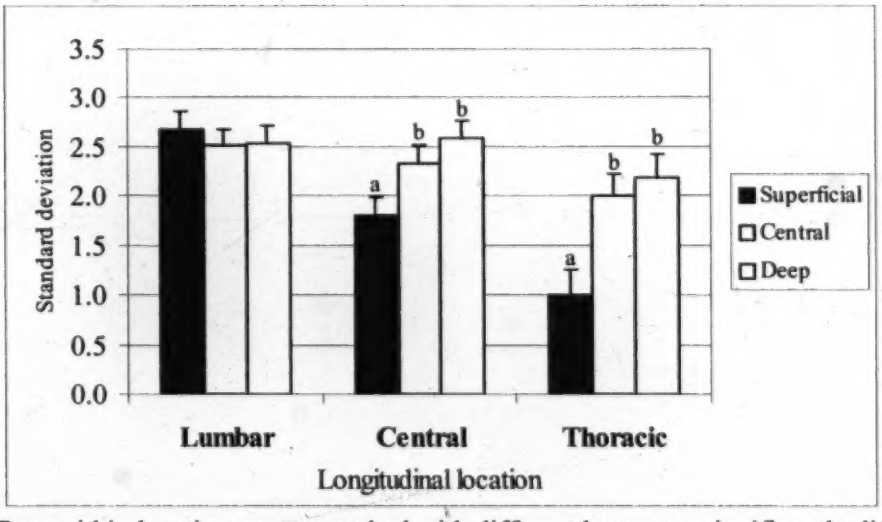


Figure 2.9. Mean shear values for each longitudinal location across the superficial-deep cross section of the *Longissimus thoracis et lumborum*. Vertical bars are standard errors.



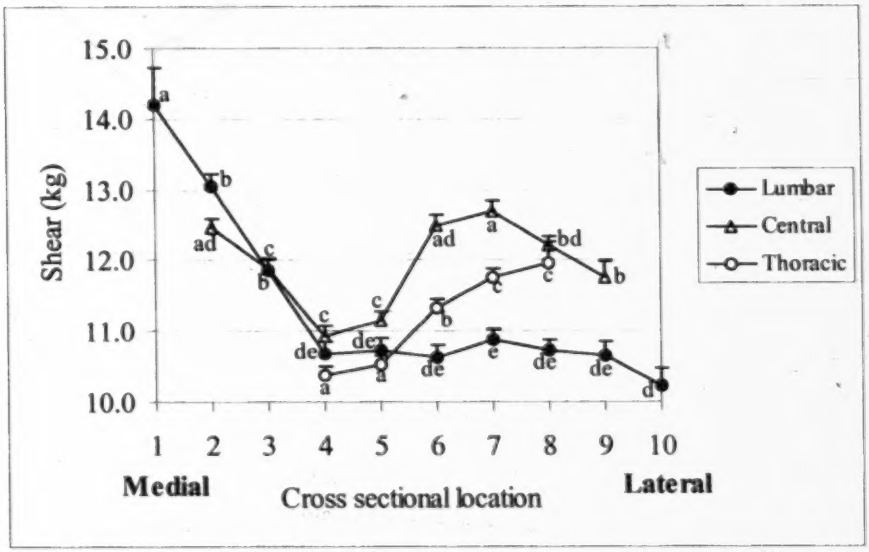
a,b,c Bars within location groups marked with different letters are significantly different ($P<0.05$)

Figure 2.10. Standard deviation for each longitudinal location across the superficial-deep cross section of the *Longissimus thoracis et lumborum*. Vertical bars are standard errors.



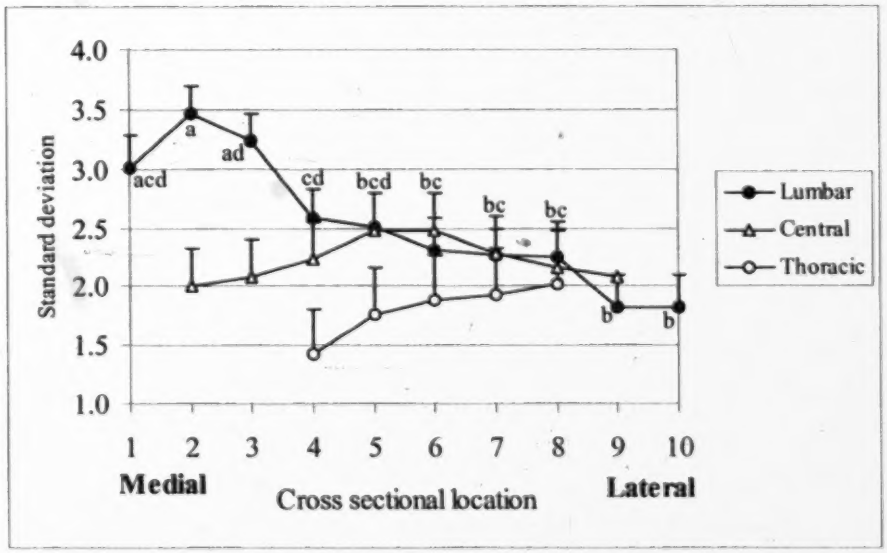
a,b,c Bars within location groups marked with different letters are significantly different ($P<0.05$)

Figure 2.11. Mean shear values for each longitudinal location across the medial-lateral cross section of the *Longissimus thoracis et lumborum*. Central and thoracic lines are centred to approximate the common mid-point in the cross section amongst the lumbar, central, and thoracic segments. Vertical bars are standard errors.



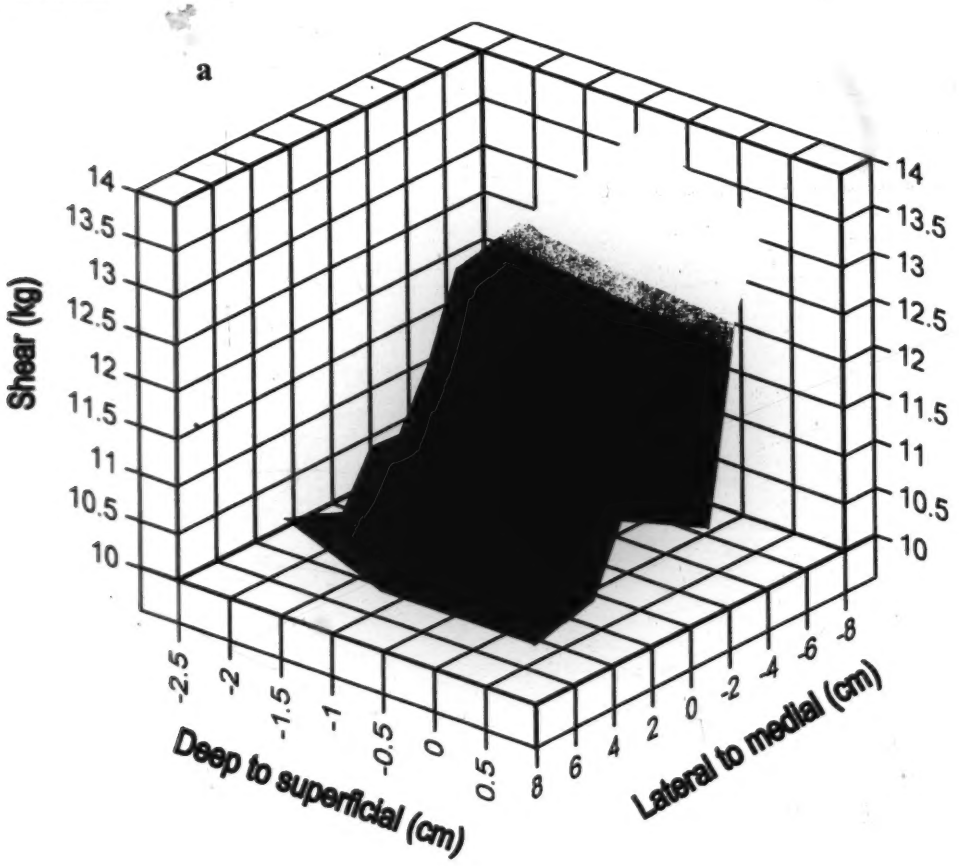
a,b,c,d Legend symbols within a series marked by different letters are significantly different ($P<0.05$)

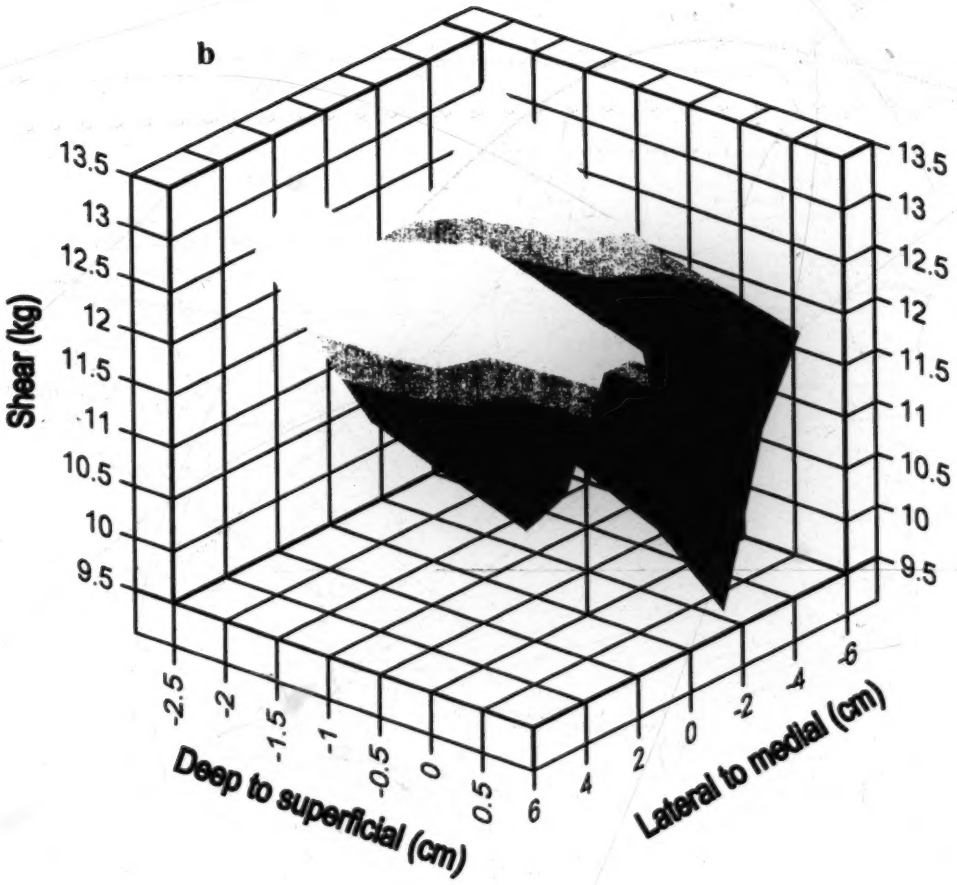
Figure 2.12. Standard deviation for each longitudinal location across the superficial-deep cross section of the *Longissimus thoracis et lumborum*. Central and thoracic lines are centred to approximate the common mid-point in the cross section amongst the lumbar, central, and thoracic segments. Vertical bars are standard errors.



a,b,c,d Legend symbols marked by different letters are significantly different ($P<0.05$)

Figure 2.13. Surface plots representing cross sectional shear values in the lumbar region (**a**), the central region (**b**), and the thoracic region (**c**) of the *Longissimus thoracis et lumborum*




b
Shear (kg)
13.5
13
12.5
12
11.5
11
10.5
10
9.5
-2.5
-2
-1.5
-1
-0.5
0
0.5
Deep to superficial (cm)
6
4
2
0
-2
-4
-6
Lateral to medial (cm)

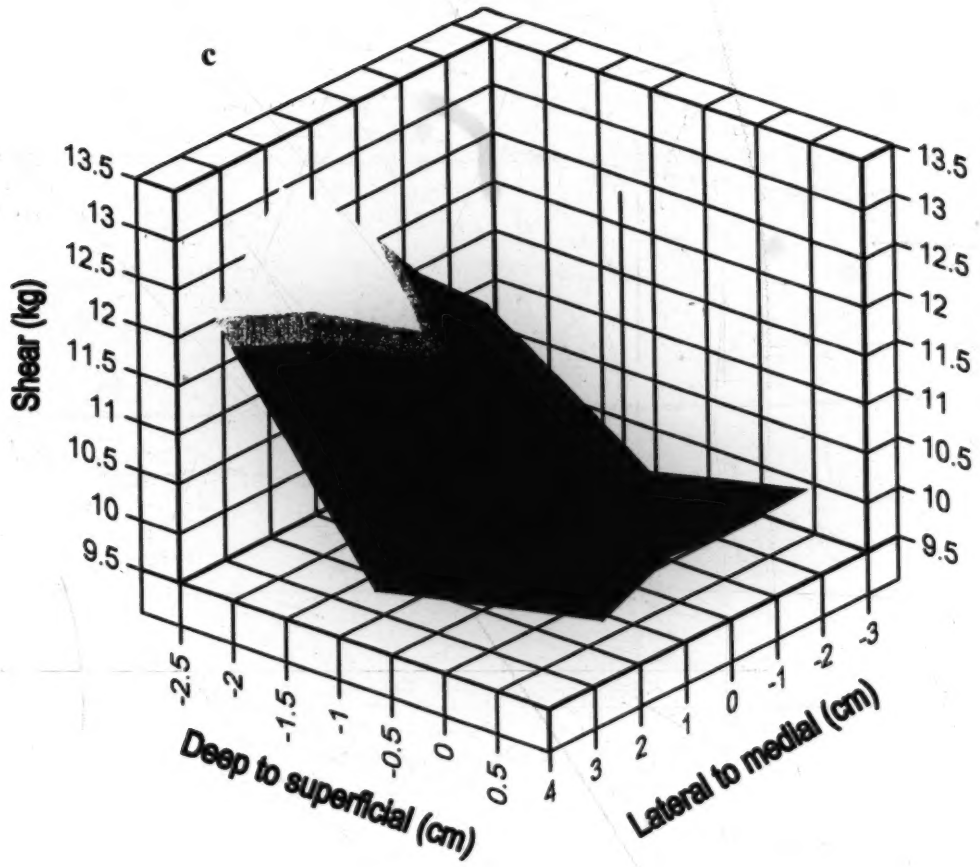
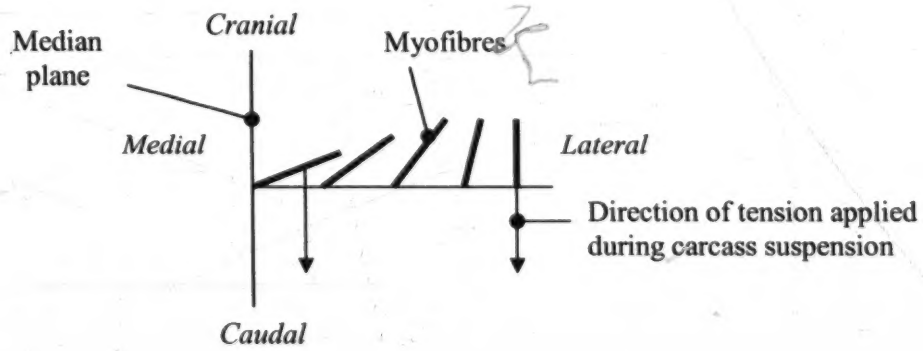


Figure 2.14. Approximation of muscle fibre angles in the medial to lateral cross section of the lumbar region of the *Longissimus thoracis et lumborum*.



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Chapter Three

Intervention strategies for enhancement of instrumental tenderness in the *Longissimus thoracis et lumborum*

A portion of this chapter has been submitted for publication. Janz, Aalhus, Robertson, Dugan, Larsen and Landry. Canadian Journal of Animal Science. In review.

3.1. Introduction

Toughness and variability of beef tenderness are key meat quality issues, yet elaborate, expensive, and highly technical solutions need not be the sole remedies for these quality problems. For the *Longissimus*, in particular, uncomplicated techniques such as altered carcass suspension, chilling modifications, and ageing may be sufficient to enhance tenderness.

Extensive, three dimensional characterisation of Warner-Bratzler shear force in the entire *Longissimus thoracis et lumborum* (LTL) (Chapter Two) indicated significant variability in instrumental tenderness values. This trend was particularly evident in the medial to lateral cross-sectional plane of the lumbar region, possibly due to variable tension applied across the LTL during conventional carcass suspension. Additional investigation of the LTL by dissection revealed a diverse pattern of cross-sectional muscle fibre arrangement as opposed to a more uniform array in the thoracic area. Berry (1993) reported unspecified, inherent properties of the lateral portion of the *Longissimus* that resulted in a uniqueness of this location in terms of tenderness/toughness. This inherent property may simply be sarcomere length.

Toughness is greatly influenced by the nature of muscle attachment to the skeleton and the extent of stretch placed on muscles during carcass suspension (McCrae et al. 1971). Koohmaraie et al. (1996) reported meat toughening results from sarcomere shortening during rigor development and that in the absence of sarcomere shortening, increases in shear force are not observed. Conventional carcass suspension permits slackening of tension applied to the *Longissimus*, particularly in the lumbar portion of the muscle. In this condition, the potential is enhanced for the development of extensive sarcomere shortening as a result of rigor tension. Locker (1960) suggested that hanging

the carcass in such a way to prevent muscle shortening would improve *Longissimus* quality. Altering carcass suspension such that weight is borne by the *obturator foramen*, with the hind limb permitted to hang freely, also referred to as "aitch bone" suspension or Tenderstretch, has since been successfully demonstrated as a technique for improving tenderness (Hostetler et al. 1970; Smith et al. 1971, Bouton and Harris 1972, Hostetler et al. 1972; Sørheim et al. 2001).

While longer sarcomere length is associated with greater tenderness, observation of sarcomere length at a single point may not adequately reflect the overall contraction state of the muscle (Howard and Judge 1968). As such, the first objective of this work was to use the extensive sampling methodology developed and reported in Chapter Two to thoroughly investigate sarcomere length in the lumbar portion of the *Longissimus* and to evaluate the effectiveness of altered carcass suspension for enhancing tenderness in this portion of the loin.

The second phase of the research examined modified chilling and individual muscle ageing as additional, simple methods to ameliorate quality concerns. Traditionally carcasses have been cooled as quickly as possible in order to minimize evaporative shrink loss and maximize plant throughput (James and Bailey 1990), as well as to ensure internal product temperature declined to at least 7°C before shipping. This type of rapid chilling, while not as extreme as the processes of blast or very fast chilling, has the potential to negatively impact tenderness by the induction of cold shortening and the impairment of proteolytic enzyme function. The original impetus for a move towards more moderate (36-48 h) carcass chilling was a noted potential for maximizing marbling score and quality grade (Calkins et al. 1980; Johnson et al. 1985), however, the implications for tenderness were not examined.

The most common method utilised to improve tenderness is to allow a period of postmortem ageing (Davis et al. 1975) during which the protein structure of meat is altered by intrinsic osmotic conditions and/or enzymatic activity. Modern distribution systems, however, have resulted in extreme variability in ageing time. Gill et al. (2002) tracked boxed primal cuts through commercial distribution in Canada. On average, boxed product exited packing plants, warehouses, and retail store coolers after 4, 13, or 22 d of ageing, respectively; however, the maximum time for exiting these distribution

points was 24, 51, and 80 d. In the United States, Tatum et al. (1997) reported variability in ageing time for top loin (*Longissimus lumborum*; LL) with an average of 19 d and a range of 2-91 d. Loin steaks available for purchase with ≤ 7 days of ageing were identified as a source of tenderness problems. Brooks et al. (2000) reported post-fabrication ageing time for ribeye (*Longissimus thoracis*) in the United States averaged 39 d (range 10-67 d) and for striploin (LL) 31 d (range 5-61 d).

Additionally, there is some information indicating ageing may not be advantageous in all muscles, particularly in the *Semimembranosus* (Eilers et al. 1996), while others have indicated great potential for the use of forequarter muscles, especially the *Infraspinatus* (Smith et al. 1978). With current inventory management, some product obviously undergoes an unnecessarily long ageing time with unknown effects on tenderness. Since refrigerated storage represents a large cost to the beef industry (Ouellette et al. 1980), meat should only be aged if improvements in tenderness are realised. The second objective of the current work, therefore, was to investigate the effects of modified chilling and ageing treatments on tenderness in the *Longissimus* muscle and to compare the effects of these treatments to the results in several other muscles.

3.2. Materials and Methods

3.2.1. Experiment One: Altered Carcass Suspension

3.2.1.1.Carcass Treatment

Four beef steers (liveweight 652.1 kg ± 10.5 SEM) were procured from a local feedlot and transported to the Beef Unit at the Agriculture and Agri-Food Canada Lacombe Research Centre. All animals were provided timothy hay and water for the 4-12 days they were housed at the Beef Unit. On the morning of each of four kill dates, one animal was transported to the on-site research abattoir for slaughter and processing according to commercial specifications with the exception that the right side of each carcass was suspended by the *obturator foramen* in a manner commonly referred to as "aitch-bone suspension". Because of the manner of animal procurement in this case, left sides were reserved for conventional treatment so as not to alter carcass value. Exploratory statistical analysis of Warner-Bratzler shear data in Chapter Two, however,

indicated no significant difference between left and right sides, so the decision to apply the altered carcass suspension treatment to only the right sides was made with confidence. All sides were chilled for 24 h in a cooler maintained at an average temperature of 1.8°C. The following morning, the fat overlying the *Longissimus lumborum* (LL) was removed to facilitate the marking of the external surface of the muscle with food grade ink to identify the caudal side of each vertebra from L6 to T12, inclusive, as well as the medial and lateral edges and the mid-line, as described in Section 2.2.2. The LL was then removed and divided at four centimetre intervals. Ten steaks were prepared, noting the alignment with the vertebral markings. The central origin, determined to be the intersection of the mid-line and medial and lateral edges previously indicated on the external surface of the whole muscle section, was marked on the cranial face of each steak to facilitate consistent location sampling. Beginning with the most caudal steak, and alternating thereafter, steaks were assigned for measurement of sarcomere length or Warner-Bratzler shear force, respectively (Table 3.1). This fixed sampling pattern was similar to the assigned sampling locations used by Shackelford et al. (1997) for the investigation of shear and sensory tenderness along muscle length.

3.2.1.2. Sarcomere Length Measurement

With the cranial face upwards, each steak was scored with a double blade scalpel with the blades set 15 mm apart. Score marks began at the central origin and moved both medially and laterally to mark four intervals on either side of the origin for a total of 8 "medial-lateral zones". A 2 g sub-sample from the centre of each zone was prepared and homogenized with 20 ml of an isotonic solution (0.25M sucrose and 20mM ethylene diamine tetraacetic acid [EGTA]) for 10 seconds following the method of Aalhus et al. (1999). A wet mount slide of each homogenate was prepared and viewed under 1000x magnification using phase contrast microscopy (Zeiss Axioskop, Germany). Six images from each homogenate were captured electronically (Sony 3 CCD camera with computer connection). Image analysis (Image Pro Plus, Media Cybernetics, Silver Spring, MD) was used to analyse the repeating striated pattern (Aalhus et al. 1999; Devine et al. 1999; Tornberg et al. 2000) measured in pixels then converted to micrometres for statistical analysis.

3.2.1.3. Warner-Bratzler Shear Force Measurement

A convection oven (Model X-80E, Bakers Pride, New Rochelle, NY) was preheated to 177°C and thermocouples, entering the medial side, were place at the geometric centre of each steak to monitor internal temperature during cooking using a Hewlett Packard 34970A Data Acquisition Switch Unit (Loveland, CO) and a computer equipped with HP Benchlink software. Steaks were placed, cranial face upwards, on wire cooking racks to permit air circulation, and placed in the convection oven until an internal steak temperature of 70°C was reached. When the final temperature was achieved, steaks were removed from the oven, temperature probes removed, and each steak placed in a plastic bag and submerged in an ice bath to arrest cooking. Once steaks were cooled to a surface temperature of 30-40°C, excess purge was poured from the bags, and samples were refrigerated until the following morning when Warner-Bratzler shear measurement was conducted.

To prepare samples for shearing, the cranial surface of each steak was scored in a grid pattern, beginning at the previously marked central origin point, using a double blade scalpel with blades set at a 15 mm width. Following these surface markings, each steak was cut into strips parallel to the Y axis (Figure 2.1) with further divisions following the muscle fibre grain exposed in the strips. Four shear samples were removed on each side of the origin along the X axis and as many as possible along the Y axis (Figure 2.1). Samples were arranged on a laminated grid surface to maintain spatial orientation, then sheared on an Instron 4301 Materials Testing System (Burlington, ON) equipped with a Warner-Bratzler shear force cell and Series 9 software, and cross-head speed set to 200 $mm \cdot min^{-1}$. Peak load was recorded in kg.

3.2.1.4. Statistical Analyses

Prior to statistical analysis, right side data were mirrored to bring the medial and lateral aspects of the right and left sides into alignment. Warner-Bratzler shear and sarcomere length data were analysed according to a factorial design using the general linear model procedure of SAS (SAS/STAT 1990) including suspension treatment, steak location, medial-lateral zone, and appropriate interactions. Effects were considered significant at the 0.05 level with subsequent means separations completed with the

probability of difference option of SAS. Correlation (SAS 1985) and regression (SAS/STAT 1990) analyses were also conducted to investigate the relationship between sarcomere length and Warner-Bratzler shear force at various cross-section location of the muscle.

3.2.2. Experiment Two: Modified Carcass Chilling and Muscle Ageing

3.2.2.1. Carcass Treatment

On each of three slaughter dates 16 beef steers (522.6 kg ± 6.7 SEM) were slaughtered under simulated commercial conditions at the Agriculture and Agri-Food Canada Lacombe Research Centre abattoir. Side weights were recorded immediately prior to chilling. Alternating between left and right, paired sides were assigned to either the conventional chilling treatment (24 h chill at 0-2°C) or a modified chilling regime (24 h at 5-6°C followed by 0-2°C until 48 h postmortem).

Temperature and pH of the LL were measured (Accumet pH module/Visor Palm Pilot, Fisher Scientific, Mississauga, ON; Orion Ingold electrode, Udorf, Switzerland) on all sides at the end of the prescribed chilling period and side weight was recorded for determination of cooler shrink loss. All sides were ribbed between the 12^{th} and13^{th} ribs and exposed to atmospheric oxygen during a 20 min "bloom" period. An instrumental colour measurement was recorded at the exposed grade site using a Minolta CR-300 with Spectra QC-300 software (Minolta Canada Inc., Mississauga, ON; light source C, 2° observer angle). Three measurements were taken across the exposed *Longissimus* surface with care to avoid areas of clearly visible aggregates of connective and adipose tissues. CIE L*, a*, and b* (Commision Internationale de l'Éclairage 1978) instrumental colour values were converted to saturation (chroma = $[a^{*2}+b^{*2}]^{0.5}$) and colour (hue = arctan[b*/a*]) and averaged. At this time, designated day 0, five muscles were removed from each side: *Longissimus lumborum* (LL), *Longissimus thoracis* (LT), *Semimembranosus* (SM), *Semitendinosus* (ST), and *Infraspinatus* (IS).

3.2.2.2. Warner-Bratzler Shear Force Measurement

Muscles were cut into individual steaks (2.54 cm thickness; cut perpendicular to muscle surface), vacuum packaged and, excepting the day 0 sample which was cooked

immediately, placed in refrigerated storage (2°C) for ageing to 7, 15, 21, and 29 days. A temperature probe, connected to a Hewlett Packard 34970A Data Acquisition Switch Unit, was inserted into the geometric centre of each steak to continually monitor internal temperature using Hewlett Packard Benchlink Software. Steaks were placed on a preheated (200°C) Garland ED-30B electric grill (Condon Barr Food Equipment Ltd, Edmonton, AB), cooked to an internal temperature of 40°C, turned, and cooked to a final internal temperature of 72°C. When the final cooking temperature was reached, steaks were removed from the grill, temperature probes were removed, and each steak was placed in an individual bag then an ice bath to arrest cooking. Samples were refrigerated overnight prior to shearing. The following morning three cylindrical cores, 19 mm in diameter, were removed from each steak with a stainless steel corer, parallel to the grain of the meat, to allow shearing perpendicularly to muscle fibres. Cores were sheared using a Warner-Bratzler shear cell attached to an Instron Model 4301 Materials Testing System (Burlington, ON), equipped with Series 9 software, and operated at a crosshead speed of 200 $mm \cdot min^{-1}$. Peak load was recorded in kg.

3.2.2.3. Statistical Analyses

Meat quality data were analysed according to a factorial design using the general linear model procedure of SAS (SAS 1990) including carcass side, chilling treatment, muscle, and appropriate interactions. Within muscle effects of ageing time were analysed separately by including time in the model along with carcass side and chilling treatment. Effects were considered significant at the 0.05 level and subsequent means separations were conducted using the probability of difference option of SAS. To assess shifts in tenderness over the ageing period and within chilling treatments, the proportion of samples on either side of a predetermined "tender" threshold (<5.6 kg vs. ≥5.6 kg; Aalhus et al. 2000) was determined using the frequency procedure of SAS. Correlation analysis to assess the relationship of shear values amongst muscles was conducted using the correlation procedure of SAS.

3.3. Results and Discussion

3.3.1. Experiment One: Altered Carcass Suspension

3.3.1.1. Warner-Bratzler Shear Force

The interaction between suspension method and steak location indicated a similar overall Warner-Bratzler shear force trend, within both treatments, to that reported in Chapter Two for the lumbar segment of the *Longissimus*. Shear values increased with an increasing distance from the caudal aspect of the muscle (Figure 2.7; Figure 3.1). The altered suspension technique resulted in lower shear values at every point of comparison to the conventional treatment (Figure 3.1). Furthermore, less fluctuation between steak locations was observed following altered suspension, with a range and standard deviation of 1.29 and 2.24 kg, as compared to 3.26 and 2.89 kg using conventional suspension.

A significant interaction also existed between suspension treatment and location in the medial to lateral cross-section. Similarly to the results reported in Chapter Two, the conventional suspension method resulted in a higher shear value along the medial edge of the lumbar *Longissimus* (Figure 3.2). Aitch bone suspension acted to reduce the range of shear values between the medial and lateral extremes and to reduce shear value overall (Figure 3.2), as similarly reported by Sørheim et al. (2001).

3.3.1.2. Sarcomere Length

The significant interaction between suspension treatment and medial-lateral zone location, displayed in Figure 3.3, indicates that suspension method influences the pattern of SL in the medial to lateral cross-section. Altered suspension created a smooth curve across the cross-sectional locations, maximizing sarcomere length in the central area. At all points of comparison, sarcomere length following altered carcass suspension was significantly longer than with conventional suspension.

In Chapter Two it was speculated that the variation in fibre angle across the *Longissimus* in the medial to lateral direction resulted in variable tenderness across the muscles perhaps due to variable resistance to rigor contraction that could result in longer sarcomere length in the lateral portion, hence lower shear values. The present results (Figure 3.3) demonstrate that conventional carcass suspension does, in fact, result in significantly longer sarcomeres in the lateral aspect of the lumbar segment of the

Longissimus, corresponding with significantly lower shear value (Figure 3.2) in this region as compared to the medial side of the muscle. Aitch bone suspension functions to increase sarcomere length overall, and to reduce shear values and shear variability (standard deviation 2.89 versus 2.24 kg in conventional and altered suspension, respectively) as compared to conventional suspension.

3.3.1.3. Relationship Between Sarcomere Length and Shear Force

The relationship between sarcomere length and shear force was examined at each location within the medial to lateral cross-section. Within the conventional suspension treatment, the only significant relationship that existed was at the extreme medial location (Table 3.2). When the same analysis was conducted within the aitch bone suspension treatment, four of eight locations yielded significant relationships (Table 3.2). The increased relationship between sarcomere length and shear with altered suspension indicates that a greater proportion of shear variability is attributable to sarcomere length at the longer sarcomere lengths generated by aitch bone suspension. Smulders et al. (1990) reported longer sarcomeres were associated with greater tenderness with nearly 30% of variability in taste panel tenderness scores accounted for by variability in sarcomere length. This group also reported that the relationship between tenderness and sarcomere length deteriorated at shorter sarcomere length.

Despite a significant increase in instrumental tenderness, some of the R^2 values defining the shear/sarcomere length relationship in the current work, while also significant, seem relatively low (e.g. 0.10-0.37 with altered suspension) despite the great importance of contraction state to tenderness/toughness development. Tarrant (1998) discussed that the straightforward relationship between toughening and actomyosin bridge formation is not always reflected in the relationship between sarcomere length and toughness possibly due to 1) variability in the strength of the bond, or 2) early acceleration of tenderization that obscures the relationship.

3.3.2. Experiment Two: Modified Carcass Chilling and Muscle Ageing

3.3.2.1. Carcass Quality

Despite the elevated temperature during the first phase of modified chilling, cooler shrink loss at 24 h postmortem did not vary significantly between chilling treatments (Table 3.3), a result that may make adoption of modified chilling appealing to industry. Temperature and pH differences between chilling treatments were relatively small in magnitude, but statistically significant (Table 3.3) and likely influenced meat colour development. Modified chilling resulted in a brighter (higher L*), more intense (higher chroma) red (higher hue) meat colour as compared to the control chill treatment (Table 3.3). Slower chilling promotes an increased rate of pH decline (Janz et al. 2000) and creates conditions conducive to brighter meat colour. A comparatively lower pH results in the preferential binding of oxygen to myoglobin as opposed to its consumption by mitochondria (Renerre 1990), thus resulting in lighter, redder meat colour at the time of carcass grading.

3.3.2.2. Instrumental Tenderness

At day 0, prior to ageing, a significant interaction between chill treatment and muscle for Warner-Bratzler shear values existed due to the limited effect of chill treatment on the SM as compared to the other muscles. Muscle was a significant main effect at each ageing interval and a comparison of shear values is presented in Figure 3.4. The IS always had the lowest shear values and the SM the highest with some intermediate fluctuation in the order of LL, LT, and ST over time. Belew et al. (2003) characterized Warner-Bratzler shear in 40 beef muscles following 14 days of ageing and reported the same order of tenderness amongst the muscles tested in the current study (IS<LL<LT<ST<SM). Using tenderness categories based on confidence intervals reported by Shackelford et al. (1991) for 1.27 cm cores, Belew et al. (2003) classified the IS as "very tender" (<3.2 kg), the LL and LT as "tender" (3.2 - <3.9kg), and the ST and SM as "intermediate" (3.9 - <4.6kg).

In both the *Longissimus lumborum* and *Longissimus thoracis*, a significant interaction between ageing time and chilling treatment was noted and was a result of the large difference in shear at day 0 and the variable degree of change between chill

treatments to day 7. In both muscles, the modified versus the conventional chill treatment resulted in lower shear values measured at each ageing interval (Table 3.4; Figure 3.5), with a significant difference observed at day 0 in both muscles, and days 7, 15, and 21 in the LL. Following the rapid initial decline in shear to day 7, additional ageing time did not result in significant further reduction. Davis et al. (1975) reported that initial tenderization (7-9 days) in the *Longissimus* is rapid and prolonged ageing (>17 days) provided only minimal improvement in tenderness. Smith et al. (1978) also indicated sensory improvement was not apparent after 11 days. Eilers et al. (1996) suggested at least 12 days of ageing for the *Longissimus*, and Miller et al. (1997) recommended 14 days to improve beef tenderness consistency and to increased consumer acceptance. Prior to ageing, the modified chill treatment resulted in a significantly lower variability in shear, as indicated by standard deviation, in both muscles (Table 3.4). A minimal effect of ageing time on variability was observed (Table 3.4). In terms of ensuring tenderness, however, additional ageing time did result in a greater percentage of steaks entering the "tender" category (shear <5.6 kg; Table 3.5). In the LL, tenderness was optimized by combining modified chill with 15 days of ageing, while ageing up to 29 days was required to meet the same frequency of tenderness in the LT.

Neither chilling nor ageing had a significant effect on tenderness or its variability in the *Semimembranosus* (Table 3.4). Despite 29 days of ageing, only about 20% of samples reached the "tender" category (Table 3.5). Smith et al. (1978) suggested 11 days ageing for optimal SM tenderness. Eilers et al. (1996) reported a small improvement with time, although even after 24 days, only about 16% of SM samples had shear values less than 3.2 kg. These results highlight the variable ageing response of different muscles. As such, muscle specific ageing times are recommended for industry use.

The ageing effect was significant in the *Semitendinosus* and was unaffected by prior chilling treatment (Table 3.4). Shear values decreased to day 7 without further significant change. The frequency of modified chilled ST samples entering the "tender" category peaked at 50.0% after 15 days of ageing, while 29 days of ageing following conventional chilling was required to reach a comparable level (45.8%; Table 3.5).

Without any ageing, the *Infraspinatus* had lower shear values than more highly valued steak cuts (*Longissimus*, *Semitendinosus*) with a gradual but significant decrease

in shear over the entire ageing period (Table 3.4). Prior to ageing, 83.3% of modified chilled IS were considered "tender" and conventionally chilled IS samples reached the same level after only 7 days ageing (Table 3.5). Johnson et al. (1988) reported the IS to be among the most tender forequarter muscles and that its size would make it ideal for steak production. McKeith et al. (1985) also reported on the consistently superior tenderness and flavour of the IS, traits that may be related to its relatively high fat content (Brackebusch et al. 1991).

3.3.2.3. Correlation of Warner-Bratzler Shear Amongst Muscles

Correlation analysis of shear values amongst muscles (Table 3.6) revealed a significant relationship between the IS and LL at three times postmortem (days 0, 7, 21) but only at day 0 for the IS and LT despite a closer physical proximity between the latter two locations. Furthermore, while these relationships were significant, at no point were they greater than R=0.41 indicating that no more than about 17% of the variability in one muscle was explained by the other. The ST and LL/LT were also significantly related at day 0, but at no other time postmortem. Other than an anomaly at day 29 with the LT, the SM was never related to any other muscle. The relationship with the LT at day 29 was not confirmed by any convergence of shear between these muscles at this time postmortem (Figure 3.4).

The LL and LT were significantly related at every postmortem ageing time, yet the relationship was not perfect (100%) and declined in strength (R = 0.68-0.34) over time despite the LL and LT being two segments of the same muscle. The correlation results amongst the various muscles were not particularly surprising and tend to highlight the limited predictive power for tenderness of one muscle for another. The results within the *Longissimus* further support the argument made in Chapter Two against extrapolation even within a muscle, particularly the *Longissimus*, with its large overall length.

3.4. Implications

Again, the shear force mapping technique was used effectively, in this case to determine the results of the tenderness enhancing treatment on beef *Longissimus lumborum* and the relationship of Warner-Bratzler shear force to sarcomere length. The results of this investigation also indicate that simple, uncomplicated carcass treatment methods can be employed to effectively enhance tenderness and reduce tenderness variability in the *Longissimus thoracis et lumborum*.

Although Sørheim et al. (2001) reported a large reduction (41%) in shear force with altered suspension, the present results indicate a difference between treatments well above the one kilogram threshold for consumer detection (Aalhus et al. 1999). With a 3.13 kg overall difference between treatments, aitch bone suspension reduced Warner-Bratzler shear force by 25% as compared to conventional suspension. Aitch bone suspension also decreased Warner-Bratzler shear variability, resulting in a reduction in shear range across steaks from 3.26 kg to 1.29 kg. Sarcomere length was increased by 28% following altered suspension, comparable to the 23% observed by Sørheim et al. (2001).

Beef sides subjected to the modified chilling regime did not incur extra shrink and developed a brighter lean colour (increased L*) as compared to control chilled sides. The modified chill treatment reduced the mean shear value across all muscles. In the LL and LT, these early effects of carcass chilling persisted throughout the ageing period, effectively reducing ageing time by a full seven days. Neither modified chilling nor ageing could be relied upon to produce guaranteed tender meat in the SM and ST. Tenderization did not appear to be a linear function of ageing time and the reaction amongst muscles was variable. Cut specific ageing times could reduce chilled storage costs in muscles, such as the SM, where extended ageing has no additional benefit to tenderness.

Despite its availability to the industry since the 1970s, Tarrant (1998) classified the Tenderstretch or aitch bone suspension method as an emerging technology. Although it has taken about 25 years to establish, this technique is now widely used in Britain and Ireland due to an increased demand for tender products and the decreased concern regarding altered primal cut shape (Tarrant 1998). Sørheim et al. (2001) reported, and it

was casually observed in the present study, that Tenderstretch resulted in unusual shapes of primal cuts in loin and round. Furthermore, elongation of the *Longissimus* may result in smaller steaks. Tarrant (1998) discussed that these issues may not be a substantial concern since consumer preference is shifting towards smaller, pre-packaged retail portions. Jeremiah et al. (1984), however, concluded that altered suspension was not a promising technique due to excessive labour cost and cooler space requirements of Tenderstretch in exchange for small improvements in tenderness. While the present results, along with many previously published reports, indicate that the improvements in tenderness following altered suspension are both significant and substantial, the preconceived notion about extra expense may explain the resistance to the implementation of the method in the North American beef industry.

Devine et al. (1999) presented an interesting discussion related to what they referred to as the two most important aspects of tenderisation: degree of shortening and modification of enzymes responsible for tenderisation. The total pre-rigor temperature history of the meat affects these components of tenderisation and cannot be easily compensated for in the post-rigor period. Tenderness variability, therefore, can be explained by the effect of temperature, particularly during rigor development, on shortening and enzyme activity. Devine et al. (1999) recommended that muscles enter rigor between 10 and 15°C, an optimum temperature range demonstrated in classic work by Locker and Hagyard (1963). Devine et al. (1999) emphasized that the requirements of minimal shortening and optimum rigor temperature effectively outweigh the effects of animal condition, stress, and breed related tenderness factors. The present work focused on the first part of the tenderness equation, that is shortening, by successfully attempting to maximize sarcomere length with the application of tension to the carcass by altered suspension during chilling. It may be possible that some combination of treatments (altered suspension, modified chilling, ageing) would be most effective for maximizing tenderness in the *Longissimus*.

Sørheim et al. (2001), however, reported that under modified carcass chilling conditions (10°C for 7h then 2°C to 48h postmortem), no significant differences in sarcomere length or Warner-Bratzler shear were noted with the combined application of the Tenderstretch method. While toughening at lower temperatures was counteracted by

restricting contraction, modified chilling resulted in tender meat even without additional treatment because the muscles entered rigor at beneficial temperatures and were not likely to cold shorten. Where carcass chilling must be completed within 24 h and a modified chilling regime is not possible, ageing and/or altered suspension may be applied to promote tenderness.

Since beef carries a higher price than other protein sources, the importance of a good eating experience is critical to maintaining, and preferably, improving buying trends (Brooks et al. 2000). Consumers are willing to pay a premium for beef of guaranteed tenderness (Boleman et al. 1997) and the above discussion sets out several relatively simple methods of providing such a product without excessive time or expense, but with attention to the requirements for specific muscles, particularly the *Longissimus*.

3.5. Tables

Table 3.1. Alignment of *Longissimus lumborum* steaks with vertebrae and analytical destination of each steak.

Steak number	Vetebrae[z]	Measurement
1	L[y]6	SL[x]
2	L5	Shear
3	L5/L4	SL
4	L4/L3	Shear
5	L3	SL
6	L3/L2	Shear
7	L2	SL
8	L1	Shear
9	L1/T[w]13	SL
10	T13	Shear

[z]Since vertebrae were wider than the 4 cm steaks, some overlap of vertebrae exists
[y]Lumbar; [x]Sarcomere length; [w]Thoracic

Table 3.2. Correlation and regression coefficients between sarcomere length and Warner-Bratzler shear force in the medial to lateral cross-section of the lumbar *Longissimus* following conventional and altered suspension.

	Conventional suspension			Altered suspension		
Cross-sectional location	R	R^2	P	R	R^2	P
(Medial) 1	**-0.52**	**0.27**	0.02	-0.39	0.15	0.09
2	-0.26	0.07	0.26	**-0.61**	**0.37**	<0.01
3	-0.20	0.04	0.39	**-0.46**	**0.21**	0.04
4	-0.08	0.01	0.73	**-0.49**	**0.24**	0.03
5	-0.27	0.07	0.26	-0.44	0.19	0.06
6	0.15	0.02	0.52	-0.32	0.10	0.17
7	0.20	0.04	0.41	**-0.45**	**0.20**	0.05
(Lateral) 8	0.04	0.001	0.87	-0.001	0.00	1.00

Table 3.3. Effects of conventional (CONV) and modified (MOD) carcass chilling on beef carcass quality parameters[z].

	Chilling treatment			
	CONV	MOD	SEM[y]	P
Cooler shrink loss ($g \cdot kg^{-1}$)	11.7	11.5	0.21	ns[x]
Temperature (°C)	5.05	7.95	0.09	<0.05
pH	5.63	5.53	0.01	<0.05
L*	35.3	37.0	0.23	<0.05
Chroma	21.7	23.5	0.22	<0.05
Hue	23.6	24.8	0.15	<0.05

[z]Within both chilling treatments, cooker shrink, temperature, and pH were measured at 24 h postmortem. All other parameters were measured at the time of carcass grading following the completion of chilling; 24h for CONV and 48h for MOD
[y]Standard error of the mean
[x]ns = Non-significant

Table 3.5. Percentage of *Longissimus lumborum* (LL), *Longissimus thoracis* (LT), *Semimembranosus* (SM), *Semitendinosus* (ST), and *Infraspinatus* (IS) samples from control (CON) and modified (MOD) chilling treatments and across the ageing period with Warner-Bratzler shear values <5.6 kg.

		Ageing time (d)				
Muscle	Chill	0	7	15	21	29
LL	CON	4.2	58.3	62.5	62.5	79.2
	MOD	29.2	79.2	95.8	91.7	95.8
LT	CON	8.3	54.2	75.0	62.5	83.3
	MOD	37.5	79.2	66.7	83.3	95.8
SM	CON	0.0	4.2	16.7	16.7	20.8
	MOD	12.5	8.3	8.3	12.5	16.7
ST	CON	16.7	33.3	29.2	33.3	45.8
	MOD	25.0	37.5	50.0	20.8	50.0
IS	CON	45.8	83.3	83.3	91.7	95.8
	MOD	83.3	70.8	75.0	91.7	95.8

Table 3.4. Comparison of Warner-Bratzler shear (kg) and shear standard deviation values between control (CON) and modified (MOD) chilling treatments and across postmortem ageing times in the *Longissimus lumborum* (LL), *Longissimus thoracis* (LT), *Semimembranosus* (SM), *Semitendinosus* (ST), and *Infraspinatus* (IS).

	Chill treatment			Ageing time (d)						P		
	CON	MOD	SEM	0	7	15	21	29	SEM	C[z]	T[y]	C*T[x]
Shear (kg)												
LL	6.15	4.78	0.13	7.73a	5.39b	4.71c	4.76c	4.74c	0.20	***	***	***
LT	5.93	5.05	0.12	7.44a	5.34b	5.02bc	5.05bc	4.60c	0.19	***	***	**
SM	7.83	7.82	0.19	8.41	7.73	7.98	7.55	7.45	0.31	ns	ns	ns
ST	6.37	6.19	0.12	7.01a	6.35b	6.11b	6.10b	5.82b	0.19	ns	**	ns
IS	4.84	4.60	0.13	5.59a	4.96b	4.65c	4.35cd	4.04d	0.21	ns	***	ns
Standard deviation												
LL	1.29	0.90	0.07	1.62a	1.05b	0.93b	0.96b	0.90b	0.11	***	***	*
LT	1.09	0.89	0.06	1.20a	0.97a	0.92a	1.03a	0.84b	0.10	*	ns	ns
SM	2.17	2.08	0.14	2.25	2.06	2.54	2.01	1.77	0.23	ns	ns	ns
ST	1.10	1.00	0.08	1.15	0.94	0.98	1.14	1.04	0.13	ns	ns	ns
IS	1.33	1.18	0.12	1.42	1.19	1.50	1.01	1.14	0.18	ns	ns	ns

[z]C = Chill treatment
[y]T = Ageing time
[x]C*T = chill by ageing time interaction
a,b,c,dWithin the ageing time effect, values in the same row followed by different letters are significantly different
*, **, *** Significant at P<0.05, P<0.01, P<0.0001, respectively
ns = non significant

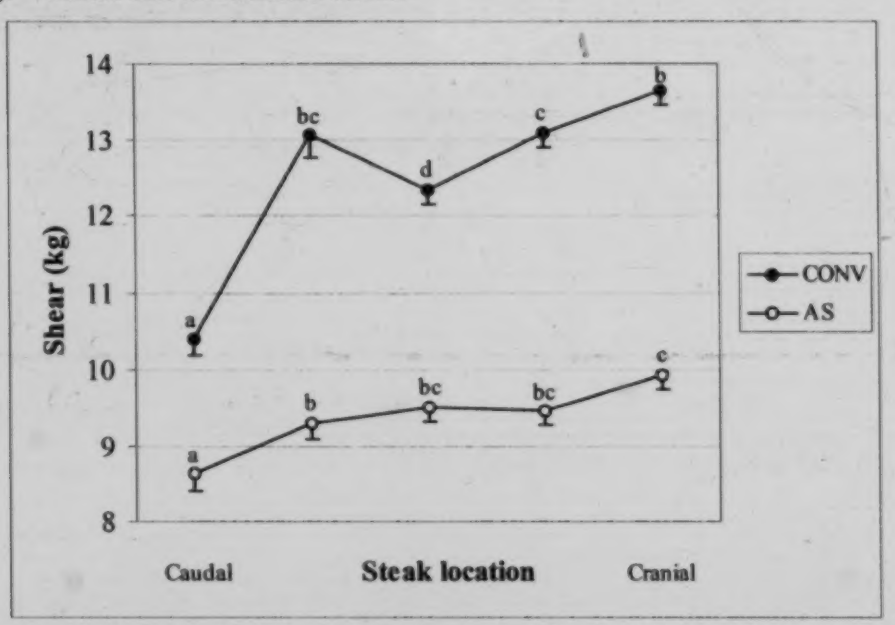
Table 3.6. Correlation coefficients (and P value) amongst shear values of various muscles at several postmortem ageing times.

			Day 0	
	LL[z]	LT[y]	SM[x]	ST[w]
IS[v]	**0.4093**	**0.3016**	0.1689	0.2787
	(<0.01)	(0.04)	(0.25)	(0.06)
LL		**0.6809**	0.0657	**0.4515**
		(<0.01)	(0.66)	(<0.01)
LT			0.0610	**0.3060**
			(0.68)	(0.03)
SM				0.1770
				(0.23)
			Day 7	
	LL	LT	SM	ST
IS	**0.3358**	0.1494	-0.0216	0.1721
	(0.02)	(0.31)	(0.88)	(0.24)
LL		**0.5763**	0.1060	0.0762
		(<0.01)	(0.47)	(0.61)
LT			0.0543	0.1085
			(0.72)	(0.46)
SM				-0.1582
				(0.28)
			Day 15	
	LL	LT	SM	ST
IS	-0.0366	0.2009	0.2616	0.0270
	(0.81)	(0.17)	(0.07)	(0.86)
LL		**0.3422**	-0.1676	0.1625
		(0.02)	(0.25)	(0.27)
LT			-0.1614	0.1580
			(0.27)	(0.28)
SM				0.0012
				(0.99)
			Day 21	
	LL	LT	SM	ST
IS	**0.3688**	0.0892	0.0615	**0.2942**
	(0.01)	(0.55)	(0.68)	(0.04)
LL		**0.3731**	-0.1091	-0.0324
		(0.01)	(0.46)	(0.83)
LT			-0.0570	0.0842
			(0.70)	(0.57)
SM				-0.0309
				(0.83)
			Day 29	
	LL	LT	SM	ST
IS	0.0311	0.1883	0.2344	0.0421
	(0.83)	(0.20)	(0.11)	(0.78)
LL		**0.3807**	0.1354	0.1199
		(0.01)	(0.36)	(0.42)
LT			**0.3471**	-0.0798
			(0.02)	(0.59)
SM				-0.0265
				(0.86)

[z]*Longissmus lumborum*; [y]*Longissimus thoracis*; [x]*Semimembranosus*; [w]*Semitendinosus*; [v]*Infraspinatus*

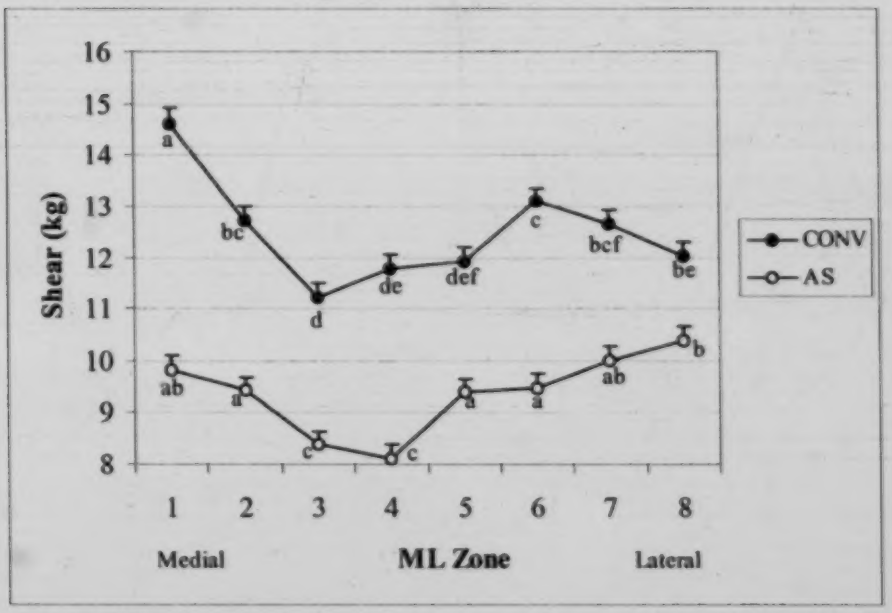
3.6. Figures

Figure 3.1. Warner-Bratzler shear value gradient in the caudal to cranial direction of *Longissimus lumborum* steaks following conventional (CONV) or altered suspension (AS). Vertical bars are standard errors.



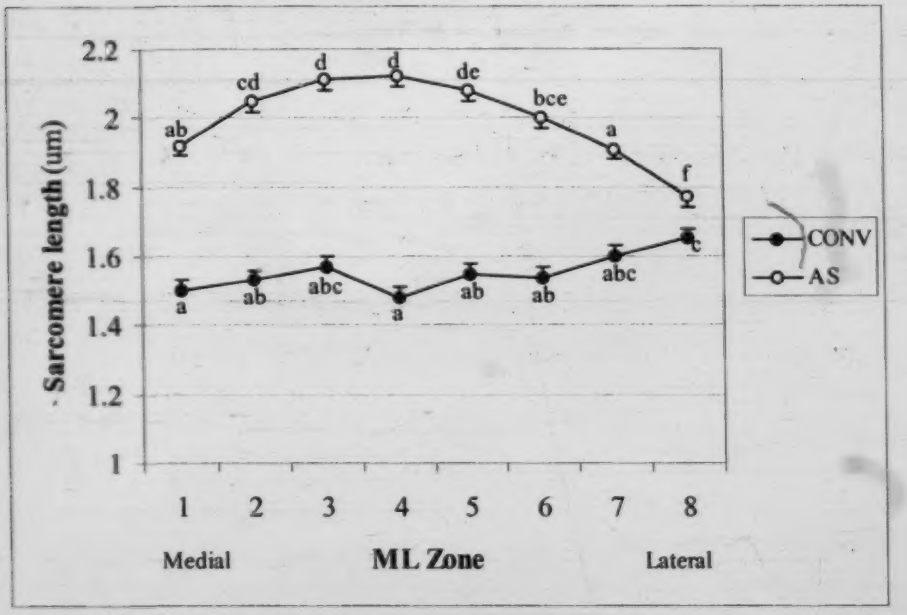
a,b,c,d Within suspension treatments, legend symbols marked with different letters are significantly different ($P<0.05$)

Figure 3.2. Warner-Bratzler shear value gradient in the medial to lateral cross-section of the *Longissimus lumborum* following conventional (CONV) or altered (AS) suspension. Vertical bars are standard errors.



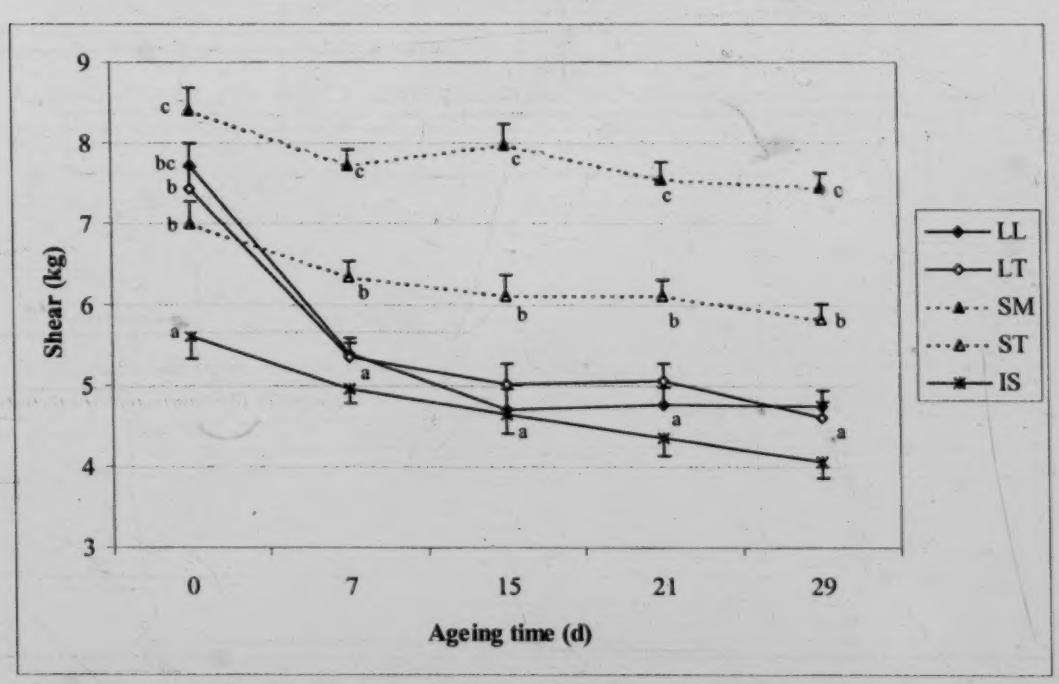
a,b,c,d,e,f Within suspension treatments, legend symbols marked with different letters are significantly different ($P<0.05$)

Figure 3.3. Sarcomere length gradient in the medial to lateral cross-section of the *Longissimus lumborum* following conventional (CONV) or altered (AS) suspension. Vertical bars are standard errors.



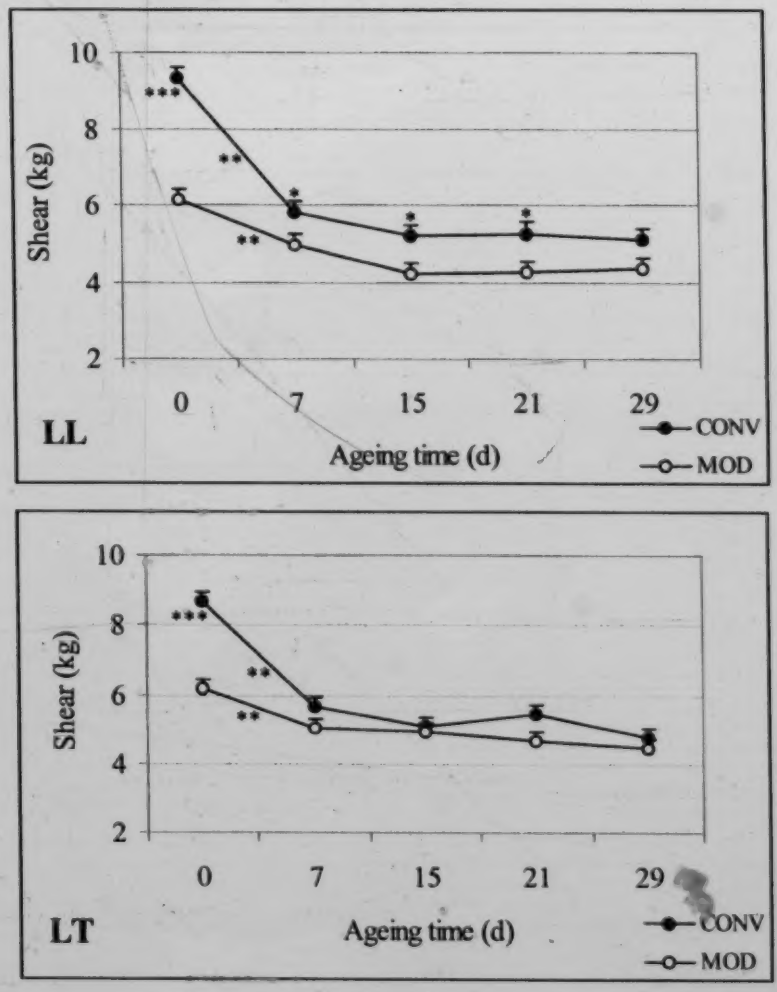
a,b,c,d,e,f Within suspension treatments, legend symbols marked with different letters are significantly different ($P<0.05$)

Figure 3.4. Warner-Bratzler shear force of *Longissimus lumborum* (LL), *Longissimus thoracis* (LT), *Semimembranosus* (SM), *Semitendinosus* (ST), and *Infraspinatus* (IS) over a 29 d ageing time. Vertical bars are standard errors.



a,b,c Legend symbols within an ageing time and followed by different letters are significantly different amongst muscles

Figure 3.5. Comparison of Warner-Bratzler shear values between chilling treatments and across ageing times in the *Longissimus lumborum* (LL) and *Longissimus thoracis* (LT). ***Indicates P<0.001 between chilling treatment; **Indicates P<0.01 between 0 and 7 days within chilling treatments; *Indicates P<0.05 between chilling treatments. Vertical bars are standard errors.



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Chapter Four

Mapping of Warner-Bratzler shear gradients in beef *Semitendinosus* and the effects of connective tissue

4.1. Introduction

According to Bailey (1972), the best quality meat, that which is suitable for grilling and roasting, is generally located in the proximal hind limb and the dorsal area posterior to the fifth rib. Unfortunately, while the rib and loin are highly marketable, the value of cuts from the round often suffers due to real or perceived problems with tenderness (Belew et al. 2003). Following a survey involving meat purchased from retail cases in 14 major cities in the United States, Morgan et al. (1991) reported that 37.5% of *Semitendinosus* (ST; eye of round) steaks received sensory scores below 5.0; that is, slightly tender on an 8 point scale ranging from extremely tender (8) to extremely tough (1). Furthermore, 56.4% of ST steaks had shears greater than 4.6 kg (1.27 cm core) that, according to confidence intervals, would result in a 50% chance of receiving a sensory panel score of slightly tough (4 on the 8 point scale).

Some mapping of tenderness/toughness trends in the *Longissimus* has been completed either in select sections (Smith et al. 1969; Martin et al. 1970; Gariepy et al. 1990; Kerth et al. 2002; Zuckerman et al. 2002) or over the entire muscle (Chapter Two). The ST, however, has been the subject of far less investigation. Henrickson and Mjoseth (1964) and Shackelford et al. (1997) examined three locations along the length of the muscle and Belew et al. (2003) and Torrescano et al. (2003) provided a mean value for whole ST for comparison to a variety of other muscles. Only Reuter et al. (2002) have made an in depth investigation, looking at average shear values across each of 11 serial sections. This type of work will provide a detailed understanding of the *Semitendinosus* and may provide insight as to its optimal use.

An important factor that could affect tenderness in the *Semitendinosus* is simply the architecture of the muscle. The ST can be described as "fusiform" with myofibres arranged parallel to the longitudinal axis of the muscle (Bailey and Light 1989) and having a single point each for origin and insertion. Furthermore, the whole muscle is

enveloped in a thick epimysial connective tissue layer. Several sources have indicated that connective tissue provides "background toughness" in meat (Bailey 1972; Stanton and Light 1990), but it may play a more direct role in the ST. Christensen et al. (2000) indicated a specific need for further investigation stating that effects of collagenous connective tissue due to shrinkage with heating cannot be ignored since it may drive water from muscle fibres and result in toughening. The specific combination of fusiform fibre arrangement and the surrounding epimysium in the ST could be the ideal conditions for excessive moisture loss from the cut ends of the *Semitendinosus* as prepared steaks are cooked.

The objectives of this work were to: 1) use the technique for thorough Warner-Bratzler shear force gradient investigation, reported in Chapter Two, for a detailed examination of beef *Semitendinosus*, and 2) to investigate the influence of epimysial connective tissue on moisture content and instrumental tenderness in the *Semitendinosus*.

4.2. Materials and Methods

4.2.1. Experiment One: Exploration of Warner-Bratzler shear gradients

4.2.1.1.Muscle Collection, Removal, and Division

Semitendinosus (ST) muscles were harvested from the same animals described in Chapter Two. Once carcass dressing to commercial specifications was completed, all adipose and connective tissues overlying the ST were removed from both the left and right sides. This served to expose the entire length of the ST between the proximal (relatively closer to the vertebral column; Frandson and Spurgeon 1992) and distal (relatively further from the vertebral column; Frandson and Spurgeon 1992) ends. Following trimming, temperature data loggers with probes (SAPAC Temprecord III Scientific Loggers, Sumaq Distributors, Toronto, ON) were placed along the length of the ST, at approximately mid-muscle depth, in distal, mid, and proximal locations to record internal muscle temperature during carcass chilling. In order to counteract the potential for cold shortening in the exposed muscle, carcass chilling was initiated in a cooler maintained at near 8.5°C (range 7.9-9.2°C). At 7.5 h postmortem, sides were moved to a cooler held at conventional chilling temperature (mean 0.8°C; range –0.4-2.0°C) for the remainder of the cooling period.

At 24 h postmortem, the medial side and mid-line (the mid-point between the medial and lateral sides of the muscle) of each ST were marked with different colours of food grade ink. Muscles were removed from each side of the carcass, weighed (mean weight 2.34 kg ± 0.05 SEM), and measured (mean length: 44.4 cm ± 1.12 SEM). From the muscle insertion point (extreme distal end of the muscle), approximately 6 cm of lean tissue were removed and discarded and 7 serial steaks were cut at 4 cm intervals. The remaining portion, about 9 cm at the muscle origin (extreme proximal end of the muscle), was also discarded. In order to fix a common, cross-sectional centre point across all steaks, the junction of the mid-line and medial edge, indicated previously on the external surface of the whole muscle, was marked on the proximal surface of each steak (Figure 4.1). In preparation for cooking, steaks were placed on a tray with the proximal surface upwards, labeled, and covered with plastic.

4.2.1.2. Cooking

Convection oven (Model X-80E, Bakers Pride, New Rochelle, NY) cooking was employed in order to maximize cooking uniformity while using a method that would not be unusual to beef consumers. With probes entering the medial side, thermocouples were placed in the geometric centre of each steak in order to continuously monitor internal temperature during cooking. Steaks were placed proximal face upwards on wire cooking racks, to permit air circulation, in an oven preheated to 177°C, and removed when internal steak temperature reached 72°C. Once removed from the oven, all steaks were placed in individual plastic bags and submerged in an ice bath to arrest cooking. When the steaks were cooled to 35 - 40°C, excess purge was poured from the bags and samples were arranged on trays for refrigeration (4°C) until shear testing the following day.

4.2.1.3. Shear Sample Preparation and Warner-Bratzler Shear Testing

With the proximal surface facing upwards, all steaks were divided into serial, rectangular shear samples. Using a twin scalpel with blades set 15 mm apart, the surface of each steak was scored in a grid pattern that originated at the pre-marked centre point: the 0,0 intersection of the X (steak width: medial to lateral) and Y (steak depth: superficial to deep) axes (Figure 4.1) as described in Section 4.2.1.1. Following the

surface markings, each steak was cut into strips parallel to the Y axis. Further division followed the grain of the muscle fibres. Only the non-uniform perimeter was discarded, since these areas did not yield samples of the appropriate size. Samples were arranged on a tray lined with a laminated grid in order to maintain the spatial orientation. Shearing was completed using an Instron 4301 Materials Testing System (Burlington, ON) equipped with a Warner-Bratzler shear cell and Series 9 software, and cross-head speed set to 200 $mm \cdot min^{-1}$. Peak load was recorded in kg.

4.2.1.4. Data Preparation and Statistical Analyses

In order to maintain proper spatial orientation, data from the right side muscles were mirrored such that medial and lateral locations between sides were aligned before further analysis. Exploratory analysis of location factors (general linear model (GLM) procedure, SAS/STAT 1990) indicated no significant difference between left and right sides. As such, left and right sides data were merged prior to further data analysis.

Analysis of shear data with the GLM procedure of SAS included steak, quadrant, shear sample location, and appropriate interactions in the model. Effects were considered significant at the 0.05 level and subsequent means separations were conducted using the probability of difference option. Correlation analysis was conducted using the correlation procedure in SAS to assess the strength of the relationship of Warner-Bratzler shear values amongst shear sample locations. Multiple comparison regression analysis was conducted using the regression procedure of SAS with the MaxR and Stepwise functions for the prediction of average shear force by shear sample and steak locations. MaxR permitted the determination of the best one variable model, the best two variable model, and so on, for the maximization of R^2. Stepwise performed a similar function, while maintaining a check on the significance (maximum $P = 0.15$) of the variables added to the model.

4.2.2. Experiment Two: Investigation of connective tissue influences on Warner-Bratzler shear in the *Semitendinosus*

4.2.2.1. Animal Processing

Animals used in this study were produced at the on-site beef production unit at the Agriculture and Agri-Food Canada Lacombe Research Centre (AAFC-LRC). All animals were British-type beef cattle finished on a grain/silage diet and had a mean liveweight of 611.4 kg ± 25.9 SEM at slaughter. On the morning of each of four kill dates, one animal was transported ~2 km from the beef unit to the research abattoir, also located at AAFC-LRC. Animal delivery was directly followed by slaughter with no intervening lairage time. Once carcass dressing to commercial specifications was completed, subcutaneous fat overlying the *Semitendinosus* (ST) muscles was removed in order to equilibrate the degree of refrigeration applied to the length of the muscle. Following trimming, two temperature data loggers with probes (SAPAC Temprecord III Scientific Loggers, Sumaq Distributors, Toronto, ON) were placed centrally in the left ST, to record superficial (~2 cm depth) and deep (~6 cm depth) temperature. Both carcass sides were placed in a cooler maintained at 9.6°C (range 6.3-10.5°C) until 8.5 h postmortem at which time both sides were moved to conventional chilling conditions (2.8°C, range 1.0-4.0°C). For the duration of carcass chilling, an additional temperature data logger was suspended from the left side rail hook, ~15 cm above the ST, to record cooler temperature.

4.2.2.2. Muscle Removal and Division

At 24 hours postmortem, muscles were marked as described in Section 4.2.1.1 then removed from each side. The extreme proximal and distal ends of each muscle were trimmed off and each ST was divided into 6 paired steaks, each 3 cm in thickness. One steak from each pair was stripped of the outer epimysial connective tissue layer using a scalpel to carefully excise the tissue from the underlying meat. These steaks were then referred to as "noCT" while the intact steak in each pair was labelled "CT". In order to fix a common, cross-sectional centre point across all steaks, the junction of the mid-line and medial edge was marked on the proximal surface of each steak (Figure 4.1).

Between each pair of steaks, a 2 cm thick section of muscle was removed for subsequent measurement of sarcomere length, and moisture contents in the raw tissue.

4.2.2.3. Cooked Sample Analyses

In order to evaluate the change in steak surface area during cooking, a tracing of each steak was recorded in pencil on cellulose acetate prior to cooking in a convection oven. Cooking was completed in a convection oven (Bakers Pride, New Rochelle, NY) preheated to 177°C. A thermocouple was inserted through the medial side of each steak into the geometric centre of the sample for continuous temperature monitoring during cooking using a Hewlett Packard 34970A Data Acquisition Switch Unit (Loveland, CO) and a computer equipped with HP Benchlink software. Steaks were placed on wire cooking racks, to permit air circulation, in the oven and cooked to an internal temperature of 72°C. Upon removal from the oven each steak was placed in a plastic bag and cooled in an ice bath to arrest cooking. Once cooled to 35 - 40°C, a post-cooking steak tracing was collected. The steaks were then replaced in plastic bags and refrigerated until the following day at which time Warner-Bratzler shear testing was completed on paired steaks from half of the muscles. Shear sample preparation and shearing followed the same methodology described in Section 4.2.1.3. except that the scalpel blades were set at a 10 mm width.

The remainder of the paired steaks were sub-sampled for cooked fibre area measurement. Two sample cubes (~0.5 x 0.5 x 0.75 cm) from the centre of each quadrant within each steak were prepared and affixed to cork tiles with cryomatrix frozen specimen embedding medium (Thermo Shandon, Pittsburgh, PA) such that fibre direction was perpendicular to the cork surface. Samples were covered with a plastic bag and set in a freezer at -80° to harden (~2-3 h). Once set, each mounted sample was fixed to a mircotome chuck and cooled at -35°C for about 20-30 min. Next, a working temperature of -10°C was maintained in a Tissue-Tek II cryostat unit (Miles Inc., Elkhart, IN) while 18 μm sections of sample were sliced with the microtome. Four sections from each sample were teased onto a room temperature slide and a coverslip was fixed in place. Slides were viewed at 80X magnification under phase contrast microscopy (Zeiss Axioskop, Germany). Ten fibre images from each slide were captured electronically

(Sony 3CCD camera with computer connection, Sony of Canada, Toronto, ON) and the minimum (d) and maximum (D) diameters of each cell were measured using image analysis software (Image Pro Plus, Media Cybernetics, Silver Spring, MD). Area (μm^2) was calculated according to: area = $\pi/4$ x d x D (Clancy and Herlihy 1978).

The remainder of the steaks used for cooked fibre area measurement were homogenized for 10 sec in a Blixer BX3 food processor (Robot Coupe USA, Ltd. Jackson, MS) and weighed into pre-weighed stainless beakers. Beakers were placed in a drying oven at 105°C for 24 h. The beakers were then re-weighed to permit calculation of weight loss representative of moisture content.

Digital images of pre- and post-cooking steak tracings were captured under a video camera (Sony 3CCD) with a computer connection and an image field calibrated for accurate linear measurement. Tracing areas were analysed using Image Pro Plus and the percent change following cooking was calculated.

4.2.2.4. Raw Sample Analyses

Each raw sample slice was divided into four quadrants as previously indicated (Figure 4.1). From approximately the centre of each quadrant, and with care to avoid large deposits of connective tissue, a 2 g sub-sample was removed and homogenized with 20 ml of an isotonic solution (0.25M sucrose and 20mM EGTA) for 10 seconds following the method of Aalhus et al. (1999) for the preparation of meat for sarcomere length measurement. A wet mount slide of each homogenate was prepared and viewed under 1000x magnification using phase contrast microscopy (Zeiss Axioskop, Germany). Ten images from each homogenate were captured electronically (Sony 3 CCD camera with computer connection). Image analysis (Image Pro Plus, Media Cybernetics, Silver Spring, MD) was used to analyse the repeating striated pattern (Aalhus et al. 1999; Devine et al. 1999; Tornberg et al. 2000) measured in pixels then converted to micrometres for statistical analysis.

Moisture content of the remainder of each raw sample was evaluated using the same method described for cooked samples.

4.2.2.5. Statistical Analyses

For statistical analysis, muscles were divided into three sections (distal, mid, and proximal) with the paired steaks in each section representative of the connective tissue treatments (CT and noCT representing those with and without epimysium, respectively). Connective tissue treatments were allocated to steaks within the sections such that both treatments were represented in all locations, and the location of the treatments was alternated between the first and second steak in each pair. The low number of muscles available for study, however, required that alternating muscles be designated for either shear analysis or fibre area/proximate analysis, both requiring the destructive use of cooked samples. As such, inferences at the steak level were not possible because of insufficient degrees of freedom for analysis.

Mixed model statistical analysis (Littell et al. 1996) was applied to all data with fixed factors including section, treatment, and where appropriate, quadrant. Data were analysed for main effects and meaningful interactions, with significance determined at the $P<0.05$ level. For analysis of moisture content in cooked samples, moisture content in raw samples was used as a covariate. The probability of difference option was used to separate least squares means of significant effects.

Principal components analysis (PCA; SAS/STAT 1990) was used to examine the interrelationship amongst shear values, steak dimension, cooked fibre area, and moisture content of cooked samples. Means for each steak were calculated for the four variables and PCA was applied to their correlation matrix. The steaks and variables were then plotted along the first two principal components which, together, accounted for 88.1% of the variability in the data set. Correlation and regression analyses (SAS 1985; SAS/STAT 1990) were conducted to evaluate the strength of the relationship between Warner-Bratzler shear and sarcomere length, overall, within sections, and by quadrant.

4.3. Results and Discussion

4.3.1. Experiment One

Along the longitudinal axis of the *Semitendinosus*, Warner-Bratzler shear was greatest in the distal section, lowest in mid region, and intermediate in the proximal segment (Figure 4.2), although the greatest difference between longitudinal locations was

only 2.21 kg with a 1.32 kg difference between the extremities. Standard deviation of shear displayed a similar trend with variability the lowest in the mid region. Investigating the "affranchi" method of muscle trimming used in France, Denoyelle and Lebihan (2003) also indicated that the extremities of the ST were less tender. When mapping tenderness in the four major muscles of the round, Reuter et al. (2002) reported that shear values in the ST were lowest in the middle and higher at the ends. Shackelford et al. (1997) drew a slightly different conclusion and reported a gradient of decreasing shear force from proximal to mid to distal, with significant differences at each level. In their experiment, however, muscles were aged 16 days and stored frozen prior to analysis. Furthermore, a Brahman influence was present in some of the animals and may have had an impact on connective tissue content and tenderness (Pringle et al. 1997).

Temperature data collected along the length of the ST during carcass cooling (Figure 4.3) indicated that the proximal and distal extremities initially chilled at a more rapid rate than did the mid section that had a greater tissue depth, perhaps decreasing the impact of refrigeration on muscle contraction during early carcass cooling.

The approximately round cross-sectional shape of ST steaks permitted the division of data into quadrants for examination of Warner-Bratzler shear trends. Data are presented in Table 4.1 and the trend is demonstrated graphically in Figure 4.4. The largest shear values were observed in the superficial-medial quadrant (3), and the lowest in deep-lateral (2), which also had the least variability. The superficial-lateral and deep-medial quadrants (1 and 4) had intermediate shears. The proximity of the deep-lateral quadrant to the musculature of the hip may have resulted in a slower cooling rate than that in more superficial locations. The greater instrumental tenderness values of the superficial locations may have resulted from a cold shortening effect on the contractile apparatus, although sarcomere length was not evaluated in this experiment.

Analysis to investigate the overall shear value relationship amongst steaks and amongst shear sample locations indicated few significant correlations (Appendix B) and no obvious pattern. Multiple variable linear regression analysis was also conducted to evaluate the predictive ability of steak and shear sample locations for the average shear values and to determine the combinations of locations required for maximizing predictive value, as indicated by R^2. All samples deemed suitable for average prediction (locations

10, 3, and 8; data in Table 4.2; locations in Figure 4.1) by the MaxR and Stepwise regression analyses were located in quadrants 1 and 4, those of intermediate tenderness. That quadrants of minimum and maximum shear values were not included was not unexpected since average prediction was the objective of this analysis. Similar results were observed with use of individual steaks for prediction of overall instrumental tenderness (Table 4.3). Steak 6, located just inside the proximal end, was the best single predictor of overall tenderness ($R^2 = 0.9436$) and other steaks from the region of intermediate tenderness where subsequently added to the prediction model to maximize R^2 at 0.9973.

Since data from the ST (Experiment One) and the *Longissimus thoracis et lumborum* (LTL; Chapter Two) were collected from the same animals, using the same method to maintain spatial orientation and to conduct Warner-Bratzler shear evaluation, a direct comparison of these muscles is valid. While the ST had lower mean and median shear values, of particular interest is the distribution of those values (Table 4.4). The standard deviation for shears in the LTL was more than twice as large as that in the ST and this is demonstrated quite clearly by the minimum-maximum range. Not surprisingly given the tighter grouping of Warner-Bratzler shear values in the ST, the number of shear samples required to predict average shear was smaller (Table 2.3-2.5, 4.2), lending an advantage in terms of extrapolating results within the muscle. Literature reports seem to vary on the ranking of ST shear against the *Longissimus*. Henrickson and Mjoseth (1964), Belew et al. (2003), and Torrescano et al. (2003) all indicated that the ST was tougher than *Longissimus* samples. In a similar comparison, though, Cross et al. (1973) observed lower shear values in the ST, but no difference between the ST and the *Semimembranosus*, *Biceps femoris*, and *Rectus femoris*. There seems to be solid agreement, however, that the standard deviation of shear values in the ST is consistently lower than all other muscles in these comparisons.

4.3.2. Experiment Two

In the presence of the an intact epimysium, cooked ST steaks had significantly greater shear values, smaller fibre areas, a larger change in steak dimension following cooking, and lower moisture content as compared to steaks with an absence of this outer

connective tissue layer (Table 4.5). Palka and Daun (1999) reported that the greatest increments in cooking loss occurred between 50 and 70°C and were the result of expulsion of water by the contracted system of myofibrillar and collagenous proteins. They also noted a significant decrease in fibre diameter at 60°C that was due to thermal denaturation of myosin and a large decrease in water retention.

Light at al. (1984) presented an interesting discussion regarded the behaviour of collagen upon heating. A general property of collagen molecules is their tendency, when heated, to shrink to one quarter of their native length and melt to form gelatin. If there are heat-stable intermolecular bridges, however, the fibre matrices shrink and develop a force rather than melting to gelatin. Higher levels of heat-stable cross-linkage leads to greater tension in the connective tissue during cooking. Light et al. (1984) demonstrated the relationship between compressive force and the amount of heat-stable cross-links. The relationship was direct and fairly linear with the *Psoas* at the bottom, the *Longissimus* in the middle, and the *Semitendinosus* near the top of the curve. As a result, Light et al. (1984) theorized that shrinkage of the perimysium and endomysium may initiate and accelerate water loss during cooking. As the myofibrillar component shrinks during cooking, water is left filling the remaining space. Once the endomysial layer surrounding each fibre begins to contract at about 50°C this fluid may be squeezed out of the cut ends of the meat. As such, the extent of shrinkage of the endomysium, the connective tissue layer surrounding each muscle fibre, directly influences the final water content of the cooked meat.

Along the longitudinal axis of the ST, a similar shear trend to that reported in Experiment One was observed; that is, the greatest instrumental tenderness was located in the mid-muscle section (Table 4.5). Cooked fibre area increased, although not significantly, with a move towards the proximal aspect of the muscle, as did the degree of steak dimension change following cooking (Table 4.5). Conversely, moisture content of cooked samples and sarcomere length increased towards the distal end of the ST (Table 4.5). Principal components analysis (PCA) permitted a closer examination of these apparent trends.

Principal components analysis is a multivariate procedure used to describe the variation in the data by the creation of a smaller set of new, uncorrelated variables, or

principal components (PCs), with a minimum loss of information (Martens et al. 1983; Sinesio et al. 1991). These PCs are "slices" through the data set, describing as large a "piece" of the total variability as possible along a single plane. The first PC describes the greatest amount of variability, followed by the second PC, and so forth in a descending fashion. The number of PCs included in the examination of PCA analysis output is determined by their individual eigenvalues. Eigenvalues indicate the relative proportion of variance in the data set described by each PC. In general, a PC with an eigenvalue greater than one is considered to be of interest (Kaiser 1960; Guinard and Cliff 1987). The analysis output (SAS/STAT 1990) also provides a correlation matrix for the original variables included in the analysis, as well as coordinate values for plotting these quality attribute vectors and individual sample locations on a biplot. In general, quality or sensory attributes are represented in a PCA biplot as vectors, while products or samples are located as points in the factorial space. Vectors have both length and direction relative to the plot origin. The length of a vector represents its variance (Couronne 1997), while the angles between vectors are equal to the correlation between the attributes. Orthogonal vectors, those at right angles, indicate divergent attributes, while colinear vectors indicate a large amount of similarity (Couronne 1997). The proximity of the sample points to attribute vectors provides an indication of the dependence of the sample on that attribute for its definition. This would be of great importance in sensory evaluation when attempting to determine the defining characteristics of a particular product. In the current work, no clearly defined clusters of samples were evident around the vectors representing quality parameters.

The PCA biplot (Figure 4.5) did, however, graphically demonstrate the inverse relationship between the moisture content of cooked samples and change in steak dimensions with cooking along the first PC. As previously discussed, a greater change in dimension and a greater moisture loss were both characteristic of steaks with intact epimysium, and the correlation matrix on which the PCA was based indicates a coefficient of correlation of -0.86 ($P = 0.0003$) between these variables. The relationship between shear and cooked fibre area along the second PC is weaker, with $R = -0.51$ ($P = 0.0938$). Cross et al. (1973) reported a lack of relationship between moisture

and shear, and all remaining comparisons amongst variables in the present work are virtually orthogonal, indicating a lack of association.

Significant results were also present within the quadrant divisions. Again, Warner-Bratzler shear values followed the same gradient as observed in Experiment One, although no literature material is available on this topic. The lowest shear values were found in the deep-lateral and deep-medial quadrants (2 and 4) and the highest in the superficial-lateral and superficial-medial quadrants (1 and 3; Figure 4.6). Cooked muscle fibre area was also significantly affected by quadrant location with larger fibre areas located in quadrants with larger shear values, and vice versa for smaller fibre areas (Figure 4.5). Sarcomere length was greatest in the deep locations (quadrants 2 and 4; Figure 4.5) the same region that had the lowest shear values. The trends amongst shear, fibre area, and sarcomere length in both the cross-sectional (quadrant) and longitudinal directions indicate that increased shear values coincide with larger fibre area and decreased sarcomere length. Taylor (2003) suggested that fibre size is the most important determinant of meat toughness. Swartz et al. (1993) discussed that the number of actomyosin rigor bonds appears to remain constant once sarcomere length is shorter than 2.7 μm. At sarcomere lengths less than 1.7 μm, the contraction state is sufficient that no I band is present and there may be longitudinal interactions between adjacent A bands that would results in a continuum of denatured myosin upon cooking.

It was speculated in Experiment One that more rapid chilling in superficial locations could have resulted in cold shortening, hence greater shear values. Examination of muscle temperature curves during carcass chilling in Experiment Two (Figure 4.7) revealed that temperature in the superficial location did, in fact, have a steeper decline than that observed in the deep location, and was lower at all times during carcass chilling. The relatively shorter sarcomere length and greater shear in these locations indicate a sensitivity of this region to ambient temperature, particularly where overlying fat tissue has been removed.

Despite the interesting overlap of sarcomere length and shear trends following analysis of each variable by quadrant, correlation analysis relationship between these variables did not reveal any sort of consistent or significant relationship. Across all shear and sarcomere length data, the correlation coefficient was –0.14 ($P = 0.18$). Data for

other comparisons of these variables by location are listed in Appendix B. Torrescano et al. (2003) also reported a small and non-significant correlation coefficient between sarcomere length and shear ($R = 0.096$, $P>0.05$). The lack of a relevant relationship between sarcomere length and shear may indicate that, although these factors may both be affected by ambient temperature, sarcomere length has very little bearing on shear values in the *Semitendinosus* and other, more dominant factors, must be considered for this muscle.

As previously discussed, Light et al. (1984) indicated that shrinkage of collagen in the peri- and endomysium initiates water expulsion with shrinkage upon cooking. This is certainly exacerbated by the fusiform arrangement of muscle fibres in the ST that presents no physical barrier to water escape from the cut surfaces of individual steaks, and the results of Experiment Two appear to support this theory. What may be a further complicating factor in the *Semitendinosus* is its unusually high content of elastin as compared to most other skeletal muscles (Bendall 1967; Nguyen and Zarkadas 1989). Elastin permits the rapid restoration of muscle tissue to its original state after deformation by muscle contraction by storing energy to facilitate this return (Bailey and Light 1989). This quality is not universal across all skeletal muscle, but the particular biomechanical function of the ST (and the *Latissimus dorsi* in the forequarter) requires a large amount of elastin. Contraction of the *Semitendinosus* results in extension of the hip joint, a movement in which energy for forward propulsion is developed. Some of this energy is stored in the elastic fibres to permit the muscle to "spring back" to its starting position once the motion has been completed (Bendall 1967).

Bendall (1967) reported that the *Semitendinosus* contained 1.82% elastin (dry matter basis) and 37% elastin as a percentage of total connective tissue, as compared to 0.19% and 5.39%, the average across 30 other muscles for these values, respectively. Furthermore, Nguyen and Zarkadas (1989) noted only trace amounts of desmosine, the indicator amino acid for elastin, in *Semimembranosus* as compared to ST. In the ST, large concentrations of thick elastic fibres are found throughout the epi- and perimysium as well as at the junctions of perimysial sheets. Finer elastin fibres are also located within these connective tissue layers. By comparison, the *Longissimus* contains less coarse elastin in the epimysium and elastin is rarely observed in the perimysium (Bailey

and Light 1989). Distribution of these connective tissue layers along the length of the ST is fairly uniform, although the content of elastin as a percentage of total connective tissue may decline towards tendons (Bendall 1967) in favour of a greater proportion of collagen at the myotendinous junctions. This uniform distribution of elastin throughout the main body of the ST may explain, in part, the relative uniformity of Warner-Bratzler shear values throughout the muscle. Furthermore, unlike collagen with its tendency to gelatinize upon heating, elastic fibres shrink and toughen (Lawrie 1989). While the amount of elastin in most muscles is very small, its intractable nature under the influence of harsh conditions demands that its contribution to toughness not be ignored (Lawrie 1989), particularly in muscles such as ST in which elastin is prevalent.

4.4. Implications

Warner-Bratzler shear force gradients in the *Semitendinosus* were thoroughly evaluated and in greater depth than has been previously reported. The mid-section of the muscle had the lowest instrumental tenderness and the proximal and distal extremities had greater shear values. Reuter et al. (2002) suggested that centre cut ST steaks could be marketed as "premium". Cross-sectional analysis indicated that superficial locations in the ST had greater shear values, possibly due to the close proximity of this region to cool ambient conditions during carcass chilling. While absolute comparisons of shear values between the ST the *Longissimus* provided varying results, the ST was clearly more uniform. A thorough understanding of the trends within individual muscles will provide insight for the development of new fabrication techniques to allow for better use of under-utilized muscles, and could provide guidance for sampling in meat quality research (Reuter et al. 2002; Belew et al. 2003).

The premise for Experiment Two was that the combination of fusiform fibre arrangement and connective tissue contraction during cooking could set up ideal conditions for moisture loss during cooking that could result in toughening of *Semitendinosus* steaks. In fact, a significant difference in shear was observed between steaks with and without epimysial connective tissue, with greater shears found in steaks with intact connective tissue. Principal components analysis, however, demonstrated that moisture loss and Warner-Bratzler shear were not related although moisture loss was a

large contributor to cooking shrinkage and the resultant change in steak dimension following cooking. Sarcomere length and shear were also not well related, leading to the conclusion that other factors must be responsible for toughness development in ST.

The most probable contributor not only to toughness, but also to the uniformity of Warner-Bratzler shear in the ST, is the unusually large proportion of elastic connective tissue is this muscle. Detailed studies of elastin in muscle have been limited not only by the difficulty of studying a virtually insoluble protein (Cross et al. 1973), but also by a common assumption that elastin plays a negligible role in meat structure and that it is mainly associated with the intramuscular vasculature. Hence, the significance of elastin in meat has been overlooked (Bailey and Light 1989). The ST is atypical of skeletal muscle with regard to its biomechanical function and resultant elastin content. As such, this muscle may best be treated by chemical or mechanical methods designed to directly effect a change in this connective tissue component in order to bring about improvements in tenderness.

4.5. Tables

Table 4.1. Least squares means and standard deviation of shear values across quadrants (see Fig. 4.1) within the *Semitendinosus*.

Quadrant	Shear (kg)	SEM	Standard deviation (kg)	SEM
1	10.74a	0.10	1.55a	0.08
2	9.67b	0.09	1.19b	0.08
3	11.83c	0.10	1.76a	0.08
4	10.71a	0.10	1.58a	0.08

a,b,c Shear and standard deviation values followed by different letters are significantly different ($P<0.05$)

Table 4.2. Regression coefficients from multiple regression using MaxR and Stepwise selection for the prediction of average Warner-Bratzler shear force from individual shear samples locations in the *Semitendinosus*.

MaxR selection			Stepwise selection	
Number of variables in best model	Shear sample location	R^2	Sample location	P
1	10	0.9968	10	<0.01
2	10, 3	0.9997	3	0.04
3	10, 3, 8	1.0000	8	0.08

Table 4.3. Regression coefficients from multiple regression using MaxR and Stepwise selection for the prediction of average Warner-Bratzler shear force from individual *Semitendinosus* steaks.

MaxR selection			Stepwise selection	
Number of variables in best model	Steak number	R^2	Steak number	P
1	6	0.9436	6	<0.01
2	4	0.9707	4	0.01
3	3	0.9854	3	0.01
4	2	0.9916	2	0.04
5	7	0.9973	7	0.01

Table 4.4. Comparison of descriptive statistics from the *Semitendinosus* (ST) and the *Longissimus thoracis et lumborum* (LTL).

	Mean	Median	SD	Min/Max
ST	10.70	10.86	1.24	7.92 – 13.33
LTL	11.49	11.16	2.54	4.59 – 23.72

Table 4.5. Least squares means for Warner-Bratzler shear, cooked fibre area, steak dimension, cooked sample moisture content, raw sample moisture content, and sarcomere length in the *Semitendinosus* by muscle section and treatment.

	Treatment				Muscle section				
	CT[z]	noCT[y]	SEM	P	Distal	Mid	Proximal	SEM	P
Shear (kg)	6.07	5.80	0.02	<0.01	6.00a	5.70b	6.11a	0.23	<0.01
Cooked fibre area (μm)	1287.27	1408.54	65.03	<0.01	1290.92	1336.56	1416.24	68.39	0.06
Steak dimension change (%)	15.84	12.63	1.71	0.05	8.08a	14.86b	19.76c	1.88	<0.01
Cooked moisture content (%)[x]	63.43	65.31	0.37	<0.01	6.61a	63.91b	62.59c	0.47	<0.01
Raw moisture content (%)	n/a[w]				73.18a	72.00b	71.59b	0.31	<0.01
Sarcomere length (μm)	n/a				2.31a	2.21a	1.99b	0.04	<0.01

[z]CT with epimysium intact
[y]no CT with epimysium removed
[x]Cooked moisture content analysis was completed with raw moisture content as a covariate
[w]Treatment not applicable to raw samples

4.6. Figures

Figure 4.1. Orientation of each *Semitendinosus* steak for rectangular shear sample preparation around a common origin (0,0) relative to the X and Y axes. Q1-Q4 indicates quadrant number and italicized numerals indicate sample numbers.

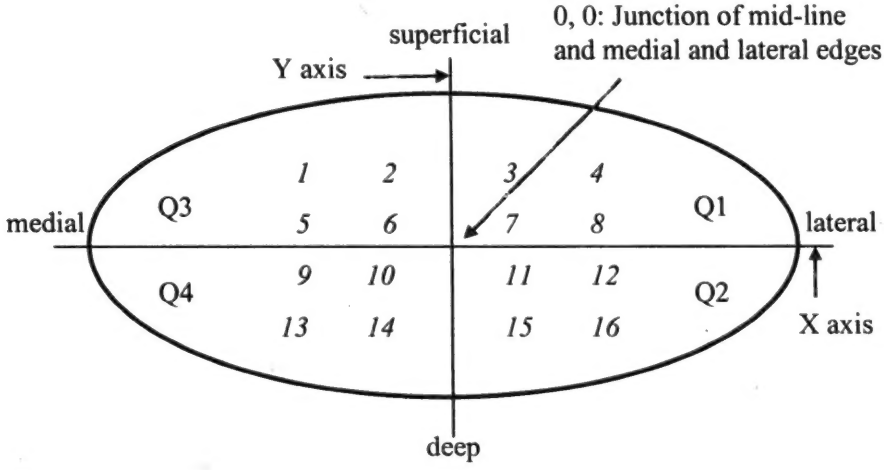
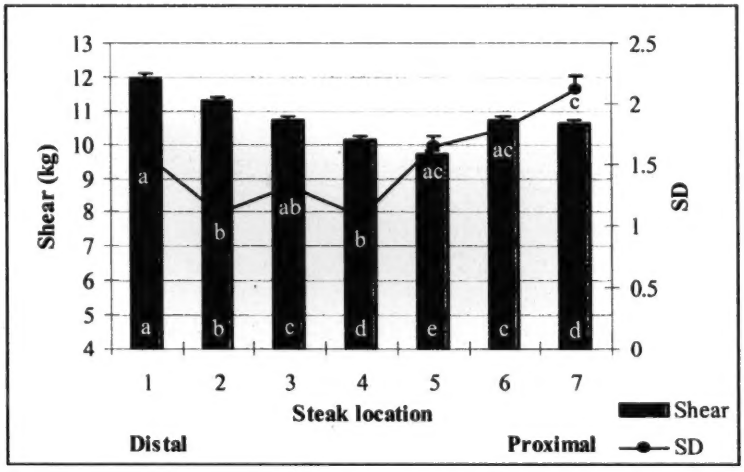


Figure 4.2. Mean shear value and standard deviation (SD) gradients across *Semitendinosus* steaks from the distal to proximal locations. Vertical bars are standard errors.



a,b,c,d,e Shear bars and SD points marked with different letters are significantly different ($P<0.05$)

Figure 4.3. Temperature (°C) during carcass cooling in distal, mid, and proximal locations along the length of the *Semitendinosus*.

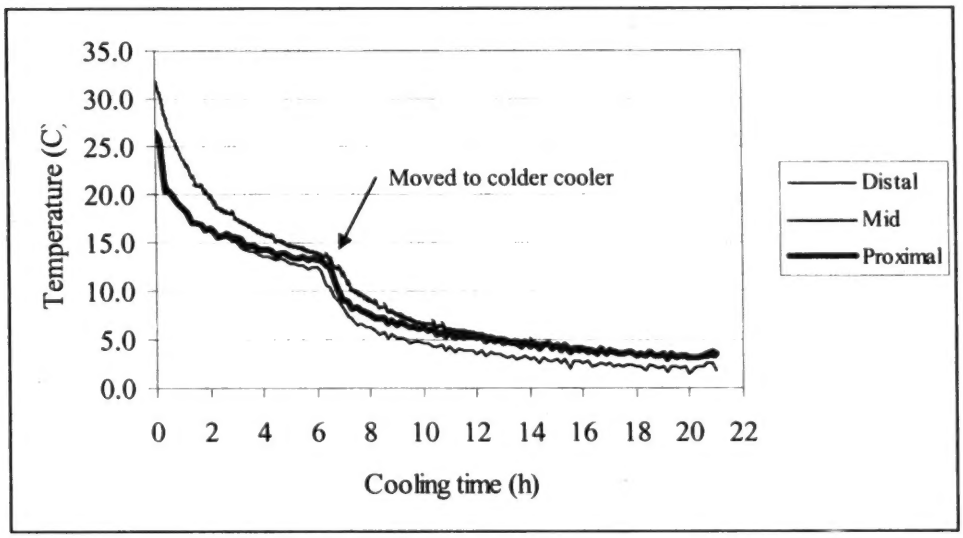


Figure 4.4. Surface plot representing shear values in the cross section of the *Semitendinosus*. Quadrants are indicated as 1, 2, 3, 4. Darker = lower shear; lighter = higher shear.

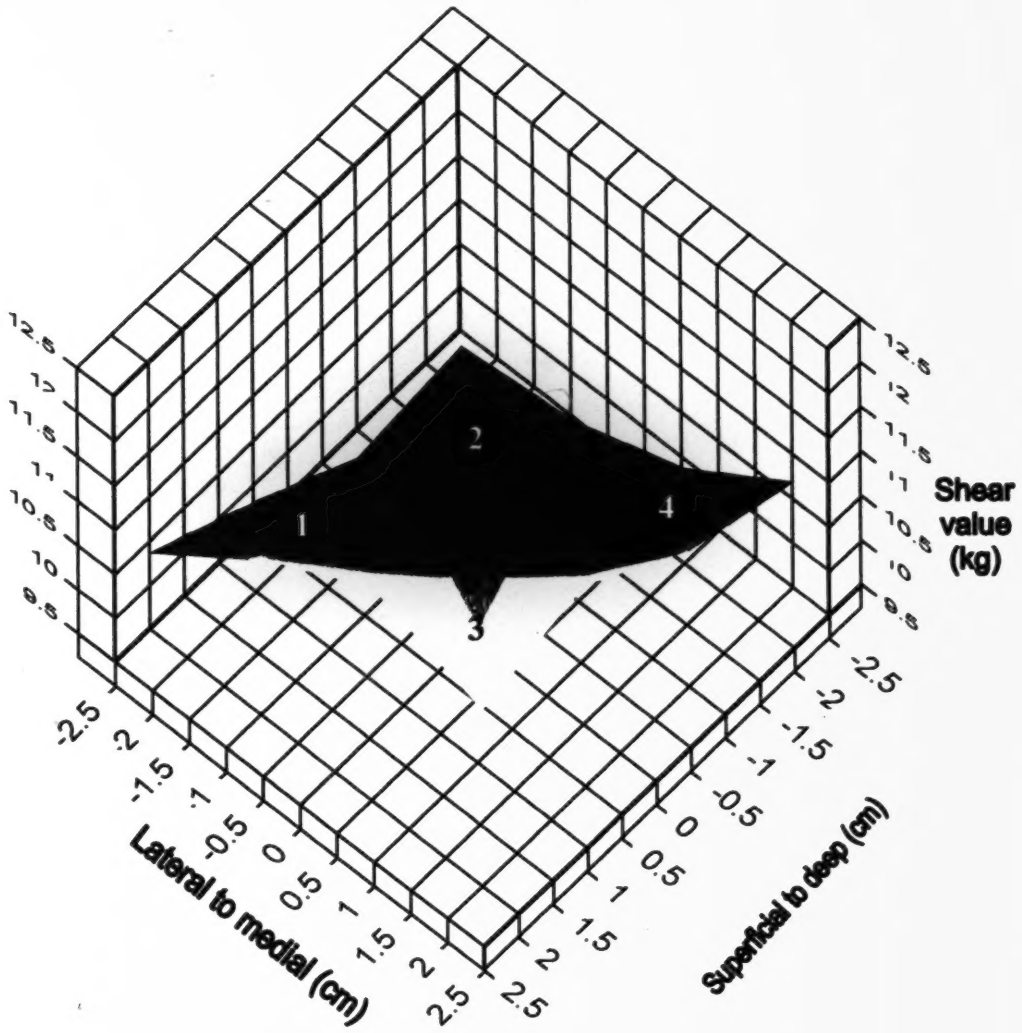


Figure 4.5. Principal components analysis biplot demonstrating the relationship amongst Warner-Bratzler shear, cooked sample moisture content, cooked fibre area, and the change in steak dimension after cooking across the first and second principal components (eigenvalues 2.08 and 1.45, respectively). Variables are represented by vectors and steaks by filled circles. Paired steaks labeled 1-6; N = noCT treatment with epimysium removed; Y = CT treatment with epimysium intact.

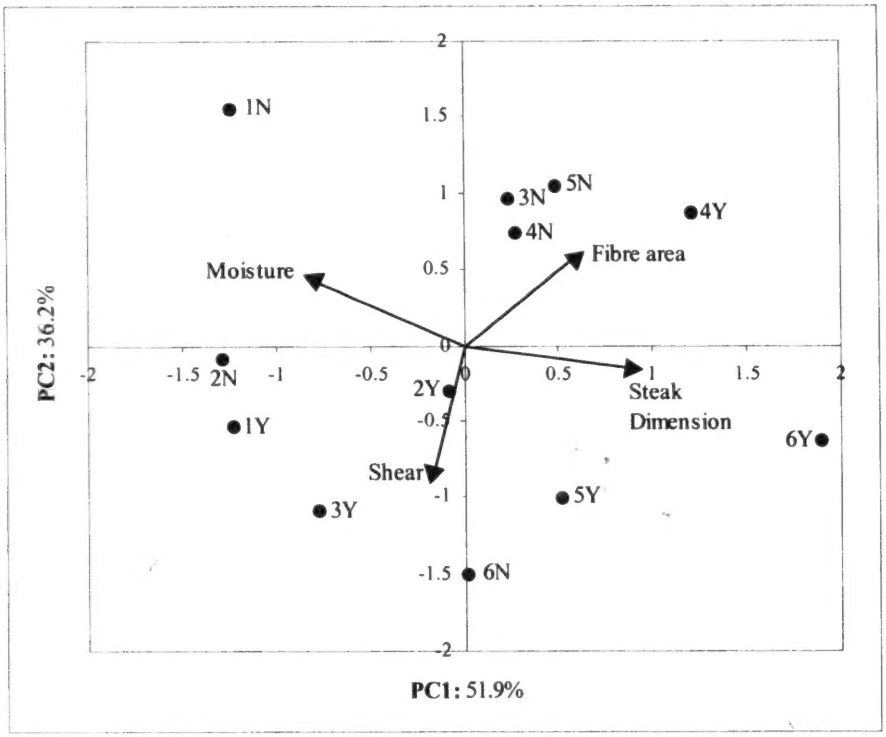
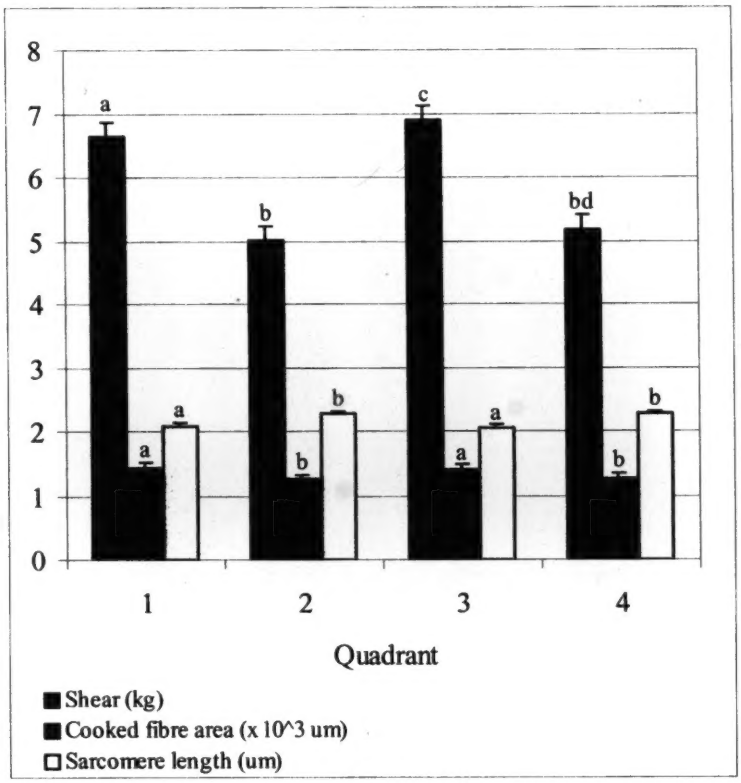
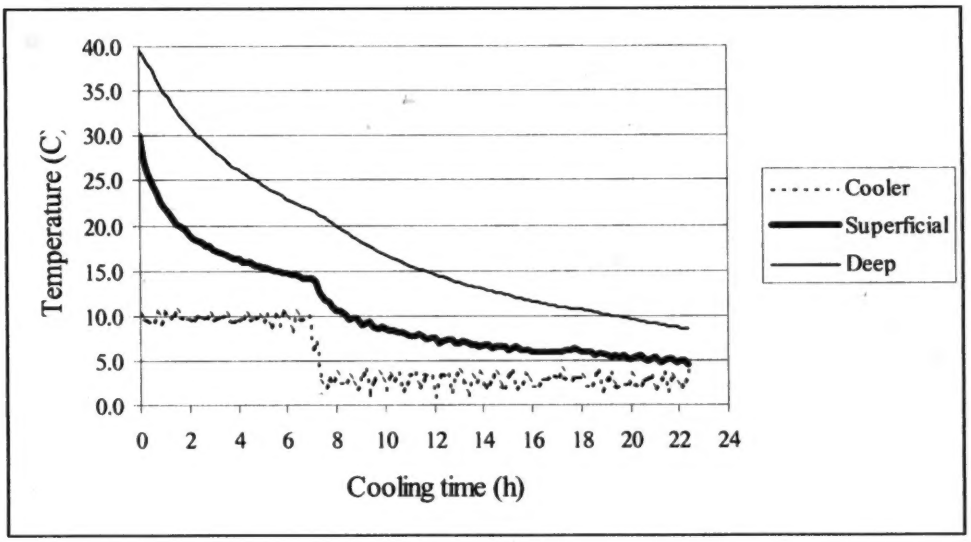


Figure 4.6. Least squares means for Warner-Bratzler shear, cooked fibre area, and sarcomere length by quadrant in the *Semitendinosus*. Quadrant 1: superficial-lateral; quadrant 2: deep-lateral; quadrant 3: superficial-medial; quadrant 4: deep-medial. Vertical bars are standard errors.



a,b,c,d Bars in each series marked with different letters are significantly different ($P<0.05$)

Figure 4.7. Temperature (°C) of cooler and superficial and deep *Semitendinosus* muscle locations during carcass cooling.



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Chapter Five

Exploration of injection treatments for enhancement of instrumental tenderness in the *Semitendinosus* from low quality, youthful beef carcasses

5.1. Introduction

While relatively simple intervention strategies can be effective for tenderness enhancement in the *Longissimus*, solving the problem of *Semitendinosus* (ST; eye of round) toughness has proven more difficult. The uniform and moderate portion size of ST steaks makes them appealing to retail consumers (Jeremiah et al. 1999), yet serious palatability concerns with cuts from this muscle have been documented (Morgan et al. 1991; Brooks et al. 2000). As discussed in Chapter Three, modified chilling and extended ageing treatments produced significant and substantial reductions in Warner-Bratzler shear force in the *Longissimus*. While the initial 7 d period of ageing significantly decreased shear values in the ST, the reduction represented a change of less than 10% and the modified chilling treatment effected no significant difference. Altered carcass suspension, also beneficial in the *Longissimus*, is not greatly effective for reducing shear force in the ST (Hostetler et al. 1970, 1972; Aalhus et al. 2000). Even a more complex calcium chloride injection treatment was insufficient to produce a tenderization effect in ST muscle from mature cows (DeYonge-Freeman et al. 2000). While this lack of effect may have been linked to the age of the animals, these authors also discussed that the negative results may not have been indicative of other muscles in the carcass and that the ST was unusual in its connective tissue attributes.

The meat consuming population is currently shifting away from traditional meal preparation and toward methods that require less time and cooking knowledge while, at the same time, maximize palatability (Prestat et al. 2002; McGee et al. 2003). This push toward more convenient products and preparation has fuelled advances in further processing with salt/polyphosphate injection treatments now commonplace, particularly in cured pork and comminuted products (Sheard et al. 1999), and mainly as a consequence of their positive effect on water-holding capacity (Molins 1991). Moisture

enhanced beef is widely available in the United States and is becoming increasingly available in eastern Canadian grocery outlets (personal communication, J. Gariup, July 2002). Its categorization as "less tender" (Boles and Shand 2001) and an unresponsiveness to more traditional tenderness enhancement treatments makes the ST an ideal subject for the investigation of suitable injection technologies.

A review of the literature indicates that moisture enhancement research, commonly applied in chicken and pork (Robbins et al. 2002), has recently become focused on beef, (e.g. Vote et al. 2000; Robbins et al. 2003) although data for the ST, specifically, are limited. Injection of a combination of ingredients, such as sodium tripolyphosphate in the presence of sodium chloride, has been demonstrated to improve less tender beef cuts and aids in meeting the demands of consumers for higher quality and convenient meat products (McGee et al. 2003).

In a study of pre-cooked beef, Boles and Shand (2001) worked with a variety of fore- and hindquarter beef cuts, injected at 110, 125, and 150% of raw weight, with brines containing salt, sugar, and sodium tripolyphosphate. Cooking yield was lowest in the *Semitendinosus*, with differences between muscles explained in part by differences in pH and connective tissue content. Shear force, however, was reduced by about 50% and shear variability decreased. Vote et al. (2000) injected beef striploin with a sodium tripolyphosphate, sodium lactate, and sodium chloride solution to 107.5-115% of original weight. Phosphate only and distilled water treatments were also applied. All phosphate/lactate/chloride treatments had beneficial effects on tenderness, juiciness, and cooked beef flavour while distilled water was detrimental to all attributes. Phosphate alone, however, resulted in a soapy flavour. Similar results were reported by McGee et al. (2003) for the *Semimembranosus*.

While salt/phosphate moisture enhancement may be beneficial in the ST, injection solutions containing proteolytic enzymes, particularly with sufficient specificity to target elastin, may also be a viable option for tenderness enhancement of this muscle. Given the possible synergism between elevated ionic strength and enzymatic proteolysis (Wu and Smith 1987), low level exogenous enzyme application in combination with a moisture enhancement solution is certainly worthy of investigation. As discussed in Chapter One, enzyme tenderization of meat is not a new concept. Treatment of meat

with enzymes breaks down structural components, reduces toughness, and produces a product with greater utility, acceptability, and profitability (Fawcett and McDowell 1987). Early studies (Wang and Maynard 1955; Weir et al. 1958) used surface application of powdered or solubilized enzymes, but a lack of uniform distribution was cited as the primary problem with enzyme use. Injection technology has essentially solved enzyme distribution problems (McKeith et al. 1994), however, many tenderizing enzymes lack substrate specificity (Takagi et al. 1992; Ashie et al. 2002). Without careful control over application and action, over-tenderization may result causing an undesirable mushy, pasty texture (Ashie et al. 2002; McKeith et al. 1994; Stefanek et al. 2002) and off-flavours (McKeith et al. 1994).

The objective of this investigation was to evaluate the efficacy of various injection treatments for the reduction of instrumental tenderness in *Semitendinosus* from low quality, youthful beef carcasses.

5.2. Materials and Methods

5.2.1. Preliminary Investigation

5.2.1.1. Enzyme Concentration and Time Before and After Cooking

Explorative preliminary work, conducted at the pilot plant for food processing in the Department of Applied Microbiology and Food Science at the University of Saskatchewan, was undertaken to narrow down the range of acceptable injected enzyme concentrations and holding times following injection. Two vacuum packaged beef *Semitendinosus* (ST) muscles of unknown origin were removed from frozen storage and thawed overnight in a refrigerated (4°C) water bath. Each muscle was trimmed of visible, external fat and connective tissues and cut into quarters, each weighing about 400 g. Due to the paucity of available food-grade, elastin specific enzymes, an enzyme "cocktail" designed for cheese making (liquid porcine pancreatin; RENCO New Zealand, Eltham, NZ; distributed by Danlac, Airdrie, AB) was chosen. In addition to elastase, the solution contained trypsin, lipase, amylase, and carboxypeptidase and, as a liquid, was easily prepared for injection application. Six injection solutions of pancreatin were formulated to provide 1%, 0.5%, 0.25%, 0.1%, 0.05%, or 0.01% in the final product when injected at 105% of raw weight. A control treatment (distilled water injection to 105% raw weight)

was also assigned such that three enzyme levels and one control were contained within each muscle. A handheld single needle syringe-type injector was used to inject samples. To reach the target injection weight, samples were injected at 6-8 individual sites. The needle was applied perpendicular to fibre direction and the solution was deposited along the injection track by applying pressure to the 60 ml syringe as the needle was slowly withdrawn. After a 30 minute equilibration period, samples were re-weighed and re-injected as needed to meet the target injection weight. Samples were then refrigerated at 6ºC. At 3 h post-injection, each section was divided into two steaks. One steak was cooked immediately and the other was further refrigerated until cooking the following day. Cooking was conducted in a conventional oven, preheated to 175ºC. Each steak was fitted with a thermocouple probe to monitor internal temperature, and cooking was terminated when this temperature reached 72ºC. Cooked steaks were placed in plastic bags, cooled in an ice bath, and immediately prepared for shear force measurement. Shear samples were prepared with a double blade scalpel with blades set 12.7 mm apart and were sheared using a TMS-90 with a CW-2 single blade meat shear cell (Food Technology Corporation, Sterling, VA). Crosshead speed was set to 200 $mm \cdot min^{-1}$ and peak force was measured in N and converted to kg.

Additional preliminary investigation was completed using meat harvested from carcasses described in Section 3.2.2. Four vacuum packaged ST muscles were removed from frozen storage and allowed to thaw at 4ºC for 48 h. All muscles were trimmed of visible, external fat and connective tissues and were divided, beginning at the proximal end, into quarters with an average weight near 470 g. Three pancreatin levels (0.02%, 0.04%, 0.06% injected at 105% original weight), slightly refined from the previous work, and a control treatment (distilled water injected at 105% original weight) were assigned to the quarter sections such that every treatment was present in every quarter location once. Using a hand-held single needle syringe-type injector, samples were injected as described above, then placed in plastic bags and refrigerated (4ºC) overnight prior to cooking. The following morning, each quarter section was cut into approximate halves prior to cooking as previously described. One steak from each quarter section was immediately prepared for shearing, as described above, while the other was placed in a plastic bag and refrigerated (4°C) for an additional 7 d prior to shearing. Shear data were

analysed by enzyme concentration, time after cooking, and their interaction using the mixed model procedure of SAS (Littell et al. 1996).

5.2.1.2. Cooking Method

Twelve STs purchased from a commercial supplier (XL Meats, Calgary, AB) and aged 6 d postmortem were trimmed of external fat and connective tissue and divided into thirds to provide proximal, central, and distal locations within each muscle. Three injection solutions were formulated to contain 0.02, 0.04, or 0.06% pancreatin in the final product injected to 105% of raw weight. Injection treatments were allocated to muscle locations such that all location/concentration combinations were present and replicated. Again, injection was completed using a hand held single needle syringe-type injector. Following initial injection, a 30 min equilibration time was permitted after which samples were re-weighed and re-injected where necessary to reach the target injection level. Samples were then placed in plastic bags and refrigerated (3°C) overnight (~18 h) prior to cooking.

The following morning, muscle sections were weighed to determine retention of the injected solutions and cut into approximately equal halves, one for grilling and the other for convection oven cooking. In preparation for cooking, each steak was fitted with a thermocouple probe inserted into its geometric centre. Probes were connected to Hewlett Packard 34970A Data Acquisition Switch Unit (Loveland, CO) to continuously monitor internal steak temperature (Hewlett Packard Benchlink software) during cooking to a final temperature of 72°C. Steaks were placed on the grill (Garland ED-30B electric grill; Condon Barr Food Equipment Ltd, Edmonton, AB), preheated to 210°C, cooked until internal temperature reached 40°C, turned and cooked to 72°C. For convection oven cooking, the oven was preheated to 175°C, steaks were placed on wire cooking racks to permit air circulation, and were cooked to 72°C without turning. For both methods, once cooking was completed, thermocouples were removed and each steak was placed in a plastic bag and immersed in an ice bath to arrest cooking. Once surface temperature reached about 35°C, steaks were ready for shear force measurement. From each steak, 6 rectangular samples were prepared using a double blade scalpel with blades set 15 mm apart. Shearing was completed using an Instron 4301 Materials Testing System

(Burlington, ON) equipped with a Warner-Bratzler shear cell, and with crosshead speed set to 200 mm·min^{-1}. Peak force was recorded in kg. Shear data were analysed by muscle location, enzyme concentration, cooking method and their interactions using the mixed model procedure of SAS (Littell et al. 1996).

5.2.2. Injection Experiment

5.2.2.1. Muscle Collection

As part of a larger study aimed at investigating the effects of diet on conjugated linoleic acid status (Lacombe Research Centre Study Plan 2003-03: Increasing bioactive lipid content in beef through pasture and feedlot management strategies), 16 British-cross beef steers were slaughtered at the Agriculture and Agri-Food Canada Lacombe Research Centre over two kill dates. Average liveweight at slaughter was 517.9 kg ± 9.0 SEM and carcass dressing was followed by a 24 h carcass chilling period at 0-2°C after which time left sides were graded according to Canadian beef grading standards (Canadian Food Inspection Agency 1992). The nutritional history of the animals used in this experiment did not include a period of intensive feedlot finishing and animals were slaughtered after a period of pasture grazing supplemented, for some animals, with barley or sunflower seeds. As such, assigned carcass grades were amongst Canada A, Canada B1, and Canada B4 reflecting trace marbling, deficiencies in muscling, and dark red colour, respectively. Individual carcass grades and explanatory notes are listed in Table 5.1. At 48 h postmortem, *Semitendinosus* (ST) muscles from left and right carcass sides were harvested from each carcass (N = 32 muscles), labelled to maintain carcass and side identity, and vacuum packaged. All muscles were aged for 7 d at 3°C d prior to freezing for storage until further processing (5-10 weeks postmortem) in the food processing pilot plant in the Department of Applied Microbiology and Food Science at the University of Saskatchewan.

5.2.2.2. Muscle Processing and Analysis

Muscles were removed from frozen storage and allowed to thaw for approximately 84 h at 3°C prior to further processing. All muscles were trimmed of visible, external fat and connective tissue, and cut into two sections, designated distal and

proximal, to create four muscle locations: left-distal, left-proximal, right-distal, and right-proximal.

Three water-based injection solutions ("brines"; Table 5.2) were prepared: an enzyme treatment (ENZ; pH 6.97), a moisture enhanced treatment (ME; pH 7.07), and a combined moisture enhanced with enzyme treatment (MEZ; pH 7.05). Where the enzyme was incorporated, brines were formulated to contain 0.01% liquid porcine pancreatin in the final injected product. This level was based on the preliminary injection concentration results. Based on a survey of recent literature (McGee et al. 2003; Robbins et al. 2003, 2002; Boles and Shand 2001; Vote et al. 2000), moisture enhanced brines were formulated to contain a final concentration of 0.5% sodium chloride and 0.25% sodium tripolyphosphate (Curafos STP, Rhodia Food Ingredients, Cranbury, NJ). Brines were mixed on the afternoon prior to use and stored in a cooler (-1.5°C) overnight to allow for temperature equilibration. Meat injection to either 105% or 110% original weight was completed using a Reiser Fomaco multineedle injector (Model FGM 20/40, Fomaco Reiser Ltd., Burlington, ON). To achieve injected weights targeting 105% of original, the needle bank was set to 52 strokes per minute at a pressure of 0.6 bar. Injection to 110% of raw weight was achieved with 34 strokes per minute at 0.8 bar pressure.

Within each injection level (105% and 110%), injection treatments, including an uninjected control (CON), were allocated to muscle locations such that each location/injection treatment combination was present and replicated. Muscle sections were injected near target levels in a single pass through the injector and allowed to sit for 15 minutes before rechecking weights. If additional injection was required to meet the target it was completed using a four needle hand injector (31 Gunjet, Spraying Systems, Co.). All samples were then vacuum packaged and refrigerated (4°C) overnight prior to shipping back to the Lacombe Research Centre for analysis.

At approximately 48 h post-injection, samples were removed from packaging and weighed to determine the retained injection level ({wt at 48 h/raw wt} x 100) and the weight change to 48 h post-injection ({raw wt- 48 h wt}/48 h wt x100). Instrumental colour parameters (L*, chroma, hue; Minolta CR-300 with Spectra QC software, Minolta Canada Inc., Mississauga, ON; light source C, 2° observer angle) and pH (Hanna

HI9025C meter with spear-type probe, Laval, PQ) of each sample were also measured. Each section was then divided into four 2.54 cm steaks and the weight of each steak was recorded.

Two steaks from each section were cooked to an internal end-point temperature of 72°C and the remaining two to 77°C using the electric gill and cooking method described in Section 5.2.1.2. These two temperatures were chosen to approximate cooking to "medium" and "well" degrees of doneness (Beef Information Centre 2003). Steaks were weighed after cooking for determination of cooking yield, placed in plastic bags, and cooled in an ice bath prior to preparation for shearing. Within each location/injection treatment combination, one steak from each end-point temperature was prepared for shearing perpendicular to fibre direction (a measure of the combined strength of myofibrillar and connective tissues; Purchas and Grant 1995) and the other for shearing parallel to fibre direction (a measure of connective tissue strength; Nottingham 1956; Christensen et al. 2000). Samples for perpendicular shearing were prepared as previously described in Section 2.2.4. using a double blade scalpel set at a 15 mm width. To begin preparation of samples for parallel shearing (Figure 5.1), steaks were scored the with double scalpel, then divided into strips parallel to the Y axis. Each strip was then laid flat to expose fibre direction on the newly exposed inner surface of each of the initial strips. The double scalpel was then used to score a 15 mm wide strip along the length of this internal face, that is the exposed side of the initial strip as defined by the cooked surfaces. Finally, each newly created "internal strip" was subdivided into 15 mm sections to form cubes that were placed in the Warner-Bratzler shear cell. All samples were sheared on an Instron 4301 Materials Testing System as described in Section 5.2.1.2.

5.2.2.3. Statistical Analyses

Treatments were arranged in a 4 x 4 x 2 x 2 factorial design including four muscle locations (left-proximal, left-distal, right-proximal, right-distal), four injection treatments (uninjected control, enzyme injection, moisture enhanced injection, and moisture enhance with enzyme injection), two injection levels (105 and 110%), and two end-point cooking temperatures (72 and 77°C) with replication. The general linear model of SAS (SAS/STAT 1990) was used to analyse the main effects and their interactions with

significance determined at the $P<0.05$ level. The probability of difference option was used to separate least squares means of significant effects.

5.3. Results and Discussion

5.3.1. Preliminary Investigation

Initial investigation into enzyme concentrations indicated no obvious difference amongst shear values following the different lengths of refrigerated storage after injection. Stefanek et al. (2002) also reported a lack of shear force difference following extended post-injection storage times of up to 4 weeks. It appeared that the enzyme complex functioned quite effectively once cooking commenced and substrate temperature increased. Cronlund and Woychik (1986) also tested the efficacy of commercially available enzymes designed for cheese making, but found them to be ineffective due to a lack of specificity for connective tissue degradation. The positive results presently observed were likely due to the choice of an enzyme complex containing elastase and trypsin, both of which are active against the targeted insoluble elastic connective tissue fractions (Kang and Rice 1970). The highest levels of injected enzyme (1% and 0.5%) resulted in proteolysis so extensive that the meat had a mushy, creamy texture and measurement of shear was not possible. At enzyme concentrations of 0.25% and 0.1%, steaks were visually unacceptable and had a sticky texture. Using 0.05% and 0.01% enzyme lowered shear values by approximately 32% and 13%, respectively, as compared to the control while maintaining acceptable appearance and texture.

In the second preliminary investigation step, both enzyme level and time between cooking and shearing were examined. Only enzyme level had a significant effect on shear and shear standard deviation, while the effect of shearing immediately after cooking versus 7 d later was non-significant. In contrast, Ashie et al. (2002) found that papain retained maximal activity even after cooking to 75°C thus increasing the risk of both texture and flavour defects. While all enzyme treatments resulted in significant 21.3-28.5% decreases in shear values compared to the control, there was no significant different amongst levels. The actual difference between 0.02% and 0.04%, however, was about 0.7 kg, nearing the 1 kg threshold for detection by a sensory panel (Aalhus et al.

1999). The difference in shear standard deviation amongst enzyme levels was significant, although no particular pattern emerged amongst treatments.

The conventional oven baking method for cooking required a variable and lengthy period of time (35-50 min) to reach final cooking temperature. The rate of heat penetration into the centres of the steaks was slow as indicated by the relatively soft texture of the centres of the samples compared to the firm outer portions in which, presumably, the enzyme was deactivated earlier in the cooking process. This defect in texture uniformity suggests that enzyme activity was prolonged in the internal areas as cooking temperature was slower to rise, hence providing an extended period of time in a temperature range conducive to optimum enzyme activity prior to thermal inactivation (Underkofler 1972). The variability in temperature penetration, hence enzyme activity, most certainly contributed to the large standard deviations observed in shear data across enzyme treatments (mean range 1.26-2.27 kg). The lack of a clear pattern in standard deviation amongst treatments may indicate that a faster, more consistent cooking method is required for enzyme injected meat.

In the final preliminary investigation step, the effects of grill versus convection oven cooking were assessed, in combination with several levels of injected enzyme, based on the assumption that these cooking methods might provide more rapid heat transfer than conventional oven baking. Despite re-injection following a 30 min equilibration period, actual injection levels were quite low. While the target was 105% of raw weight, the average actual injection level was 101.7% ± 0.15 (SEM). Boles and Shand (2001) reported that the *Semitendinosus* had the lowest brine retention amongst the selection of fore- and hindquarter muscles they investigated and were able to achieve an average injection level of only 117.4% across injection targets of 110-150%. One contributing factor to the poor retention in the present study may relate to the use of water, rather than a phosphate brine, as the carrier for the enzyme solution. Working with pork, Sheard et al. (1999) reported that target injection levels were difficult to achieve with water injection, however, phosphate-containing solutions were readily taken up and actual injection levels slightly exceeded target weights. Furthermore, with a 2 mm diameter, the openings in the injector needle were fairly large possibly permitting pooling of injected solution between muscle fibres and fasciculi. Pooling, in combination

with the direction of the ST muscle fibres perpendicular to the cut ends of the injected sections, may have set up a situation where injected solution was able to freely flow from the meat after injection.

A significant interaction was observed between enzyme concentration and cooking method (Figure 5.2). Within the grill cooking treatment, an inverse linear relationship existed between shear and enzyme level. As the concentration of injected enzyme in the final product increased, Warner-Bratzler shear force was reduced. Overall, the convection oven treatment resulted in significantly lower shears as compared to grilling, but these seemingly positive results are due in large part to serious texture defects in samples from the slower convection oven cooking method. Nearly 40% of these steaks had mushy spots and areas where meat texture was totally degraded to a paste-like consistency. Furthermore, increase in average shear value at the 0.06% enzyme level were not completely indicative of actual tenderness because many spots were too soft to prepare for shear testing. Specifically, 6 steaks cooked in the convection oven could not be sheared at all, all from within the 0.04 and 0.06% treatment groups, and 8 steaks had an abundance of soft spots which had to be avoided during shear sample preparation. In the grill-cooked group, it was possible to prepare all steaks for shearing and only five had soft spots (2 from 0.04% group and 3 from 0.06% group). Within in the grill treatment, these soft spots were likely due to uneven introduction of the injected solution with the hand-held syringe injector. Within the convection oven treatment, uneven enzyme distribution was compounded by the fact that cooking required about three times longer to achieve end-point temperature, thus permitting an extended time for enzyme activity during cooking. The faster grill cooking method was more effective for controlling enzyme action so as to permit tenderization without resulting in the textural defects caused by over-tenderization.

5.3.2. Injection Experiment

5.3.2.1. Injection Level, Weight Loss, Colour, pH, and Cooking Yield

In the ME and MEZ treatment groups, actual injection levels maintained to 48 h post-injection were very close to the 105% and 110% target levels while retained injection levels in the ENZ samples were well below the targets (Table 5.3). The results

for the ME and MEZ groups were as expected since inclusion of a phosphate in the injection solution aids in brine uptake and retention (Sutton et al. 1997). Following injection to target 105% or 110%, the weight of ENZ samples declined to 97.34% and 97.99% of raw weight, respectively, demonstrating a loss of a portion of the original sample weight in addition to that gained immediately following injection. Interestingly, there was no significant difference between the retained injection values within the ENZ treatment despite injection to meet different target levels. It appears that sample weights within this treatment retreated to some "base level" following both injection level treatments. Despite this large fluid loss, however, the ENZ treatment did not produce a weight loss as large as the drip loss observed in the uninjected CON group (Table 5.3). Sheard et al. (1999) also observed weight losses in uninjected controls and water injected pork samples although the loss following water injection was not as substantial as that from controls. Boles and Shand (2001), however, discussed differences in the injection characteristics of beef versus both pork and poultry. Casual observation of the purged fluid from all treatments indicated that in addition to a larger volume, fluid lost from ENZ samples was a darker red colour suggesting that a greater proportion of the weight lost from these samples represented water soluble sarcoplasmic proteins, including myoglobin. Injection of this phosphate-free, low-ionic strength solution ensured it was well distributed to the interior of the muscle thus increasing contact with proteins soluble under just such conditions (Molins 1991).

The improved brine retention and weight gain amongst samples injected to contain phosphate was likely due to the combined effect of physical changes to structural proteins in the presence of salt/phosphate and the elevated post-injection pH induced with this treatment (Table 5.4). Elevated pH is a common target in moisture enhancement systems (Miller 1998) and authors including Smith et al. (1984) and Robbins et al. (2002) have reported a pH increase in both beef and pork roasts following phosphate injection. Addition of an injected phosphate moves meat pH away from the isoelectric point of contractile proteins, particularly myosin (Ellinger 1972), thus increasing interfilament spacing (Offer and Trinick 1983), as evidenced by myofibrillar swelling (Bendall 1954; Xiong 1999), and effectively increasing the physical space available for water (Offer and Trinck 1983). Furthermore, the combined presence of phosphates and sodium chloride

promotes binding of oppositely charged ions to charged locations on the myofibrillar structure, thus altering the net charge of the protein (Molins 1991). The altered charge enhances electrostatic repulsion between myofilaments resulting in the creation of additional space amongst the proteins thus permitting stronger water immobilization by capillary action (Xiong 1999).

Examination of instrumental colour data shows a significant effect of both injection level and injection treatment (Table 5.4). While statistically significant, the increased L* (lightness) and chroma (colour intensity) due to injection level were not substantial enough to result in a large visual difference. The colour difference induced by the various injection treatments, however, was significant and quite visually evident. The ME and MEZ treatments that included sodium tripolyphosphate, resulted in meat with a darker, deeper red colour of lower intensity as compared to CON and ENZ. Robbins et al (2002) reported a darker colour in phosphate injected beef rounds and suggested the colour effect was a result of an increased pH. The ENZ treatment resulted in the lightest colour likely due to a large loss of myoglobin as discussed above. Additionally, ENZ samples were immersed in an abundance of purged moisture until 48 h post-injection, a condition that may have contributed to protein solubilization, thus ensuring the largest L* value amongst the injection treatments.

Cooking yield was significantly affected by the injection level, injection treatment, and cooking temperature (Table 5.5). Injection to 110% resulted in a lower cooking yield than 105% injection. This decrease was unexpected; however, Boles and Shand (2001) also reported a decline in cooking yield at a high injection level. In their report, across a variety of muscles, injection to 110% and 125% of original weight produced cooking yields of 91.8% and 93.6%, respectively, while 150% injection resulted in a cooked yield of only 84.4%. These authors suggested that the much lower ionic strength of the brine formulated to achieve target salt and phosphate levels at 150% injection may have lowered the water holding capacity in these samples. Robbins et al. (2002) also reported a greater cooking loss following 110% injection of beef rounds with a salt/phosphate brine. This group suggested that with injection, there was simply more liquid to lose during cooking, despite the enhanced water holding capacity induced by the injected brine. Cooking yield was also affected by the injection treatment with the ENZ

group experiencing the greatest weight loss during cooking, followed by the CON treatment, both treatments lacking phosphate and having lower pH than the ME and MEZ groups. Cannon et al. (1993) reported a detrimental effect of lower pH on weight loss following cooking. The ME and MEZ had the greatest cooking yields due to the ability of the injected solution to increase moisture retention. As expected, the higher end-point cooking temperature also resulted in lower cooking yield, an observation also reported by Sheard et al. (1999).

5.3.2.2. Warner-Bratzler Shear

The higher injection level (110%) decreased shear values measured in both the perpendicular and parallel directions, as well as variability within steaks (Table 5.5). McGee et al. (2003) treated beef inside rounds with three injection levels ranging from 105-109% raw weight and observed no significant difference in shear due to injection level; however, the range of treatments was narrow. Vote et al. (2000) observed a small but non-significant decrease in shear amongst beef strip loins injected at 7.5 to 15% above original weight. Boles and Shand (2001) reported a significant decrease in shear force as injection level increased from 110% to 125% across a variety of beef cuts.

In addition to myofibrillar swelling initiated by an increased pH with phosphate injection, salt and phosphate act synergistically to depolymerize myosin filaments and partially dissociate actomyosin (Offer and Trinick 1983), thus resulting in a breach of structural integrity and a resultant decrease in shear force. No significant effect of injection level on pH was observed, and salt/phosphate concentrations in the final products were designed to remain constant across both injection levels. As such, the decreased shear observed with increased injection level may simply be due to the greater availability of water for absorption into the system. The disrupted protein structure allows increased water-binding sites to be liberated (Ellinger 1972) by the extraction of myofibrillar proteins from structural architecture and their subsequent interaction with water via hydrogen bonds (Xiong 1999). Additionally, the salt ions bound to these proteins further interact with water (Molins 1991). So, additional water introduced to a system primed for water uptake and interaction may explain the decreased shear force observed with 110% injection.

The CON and ENZ treatments yielded identical perpendicular shear values (Table 5.5) indicating that a low level of enzyme suspended in a water medium that is rapidly lost as post-injection purge may not be sufficient to maintain adequate enzyme in the tissue in order to affect a shear difference. Unexpectedly, ME treatment resulted in an increased perpendicular shear. MEZ produced the lowest shear values, although not significantly lower than CON and ENZ in the perpendicular direction, suggesting that phosphate and salt in the injection solution assisted with maintenance of the enzyme within the tissue. In the parallel measurement direction, however, shear was significantly lowered by the MEZ treatment, as compared to CON and ENZ, with an overall numeric decrease across CON, ENZ, ME, and MEZ (Table 5.5).

It is possible that the ionic strength of the injected ME solution was insufficient to result in decreased shear when injected at low levels; although other workers have also used phosphate and salt levels in a range that included the present work (Papadopoulos et al. 1991a,b; Brewer et al. 1999; Vote et al. 2000; Boles and Shand 2001). It is also worth noting that casual observation of samples prepared for shearing indicated that many samples that were quite juicy upon preparation for shearing actually yielded large shear values. It is entirely possible that Warner-Bratzler shear force measurement is not the best method of analysis for an injected product since juiciness is not reflected. Perhaps sensory evaluation of tenderness, juiciness, and overall palatability would have better reflected the effects of the injection treatments. Regarding the use of exogenous enzymes, Underkofler (1972) stated that meat tenderization can be detected by sensory means long before extensive hydrolysis can be detected by chemical or histological analysis.

While the preliminary work demonstrated the effectiveness of low levels of enzyme, it is possible that the grill cooking used in the injection experiment was too rapid for the thickness of steaks employed. Owing to the size of the muscles and the number of steaks per muscle, individual steaks were slightly thinner than those used in the preliminary testing. Rapid cooking may have limited the effectiveness of the enzyme treatments by promoting rapid thermal deactivation, and obscured the effects of moisture enhancement.

Interestingly, the ME treatment produced a significant increase in shear standard deviation (Table 5.5). While, as previously noted, this may have been artifactual of the instrumental tenderness measurement method, casual observation of shear samples indicated a springy, "hammy" texture in some samples, while others were quite moist but not springy. Improved distribution of injected brines, by spray injection (Freixenet 1993; Xargayó et al. 2001) or vacuum tumbling (Boles and Shand 2002), for example, might ensure uniform texture development with the use of tripolyphosphate.

It was also interesting to note that increased end-point cooking temperature resulted in a significant decrease in shear force measured perpendicularly to fibre direction (Table 5.5) indicating an effect on the combined myofibrillar and connective tissue components. A minor but non-significant temperature effect was also noted in the parallel shear direction, that is associated primarily with connective tissue strength, with a slight decrease in shear at the higher temperature (Table 5.5). Additionally, increased end-point temperature produced a more uniform cooked product, significantly decreasing the variability across steaks, as indicated by a reduction in standard deviation (Table 5.5). Sheard et al. (1999) reported a decrease in sensory tenderness scores with a higher end-point cooking temperature of injected pork loins. Vote et al. (2000), however, reported that the shear force reducing benefits of sodium phosphate/lactate/chloride injection treatments were more pronounced at an end-point cooking temperature of 77°C versus 66°C when the differences from uninjected controls were examined. In the present work, the higher end-point temperature, therefore the increased cooking time, may have promoted increased hydrolysis of collagenous connective tissues, particularly in samples with added moisture. These hydrolytic effects could not have been expected for the elastic connective tissue component, however, since elastin does not undergo thermal transition below 100°C (Rowe 1986). Cheng and Parrish (1976) demonstrated degradation of perimysial fibres beginning at 70°C and increasing in intensity at 80°C. Furthermore, Davey and Gilbert (1974) speculated on the development of myofibrillar fragility near 80°C. While the current procedure saw samples removed from heat when temperature at the core reached 72 and 77°C, more peripheral areas of the samples may have reached temperatures above these prescribed end-points.

Finally, Warner-Bratzler shear force was significantly affected by location within the muscle. Similarly to Shackelford et al. (1997) who also used previously frozen samples, the distal ST location had lower shear values than the proximal (Table 5.5) in samples measured both perpendicular and parallel to fibre direction. While not significant, the shear standard deviation within steaks tended to be larger in the proximal versus distal location (Table 5.5).

5.4. Implications

The preliminary work and some of the trends in the injection experiment demonstrate that use of the pancreatin enzyme cocktail is a promising technique for *Semitendinosus* tenderization, particularly in combination with a salt/phosphate moisture enhancement brine. The injection experiment results were rather disappointing in that the typically large changes in Warner-Bratzler shear reported in the literature, and even in preliminary work, were not observed within the ENZ, ME, and MEZ treatments. Some positive changes were noted, however, indicating that this muscle is amenable to change with the use of injection treatments. The nature of those treatments, however, requires refinement and precise definition. While the specificity of pancreatin is quite suitable for use in meat, further work might include a range of enzyme levels paired with varying enhancement treatments and levels, and steak thickness.

Analysis of the microbiological implications of injection treatments must also be examined in detail. Kastner et al. (2001) discussed that surface contamination may be carried to the interior of products subjected to injection processes, thus increasing the risk of food borne illness. Robbins et al. (2002) concurred and reported results indicating a shorter shelf life with enhancement. Injected samples had a greater initial microbial load and shorter time to spoilage due to the invasiveness of the injection process. Bohaychuk and Greer (2003) and Glaeser et al. (2003) reported slightly higher incidences of *Listeria* species amongst commercially produced moisture enhanced pork loins as compared to uninjected controls. Glaeser et al. (2003) also reported the presence of *Salmonella* in one sample. Amongst pork consumers, the idea still exists that pork must be cooked to well done. While this was formerly the case due to the risk of trichinosis, nematode contamination is no longer an issue, although this cooking trend still continues and can

result in tough, dry, unpalatable meat (Smith et al. 2002). As such, moisture enhancement of pork has been successful in providing insurance against over cooking. Enhanced beef, however, may pose a significant food safety risk because whole muscle beef products tend to be cooked to a lesser degree of doneness than pork. In the present work, cooking to a final internal temperature of 77°C (well done, Beef Information Centre 2003) actually resulted in a lower shear value. As such, any risk of product contamination by injection need not be associated with a risk of reduced palatability if elevated cooking temperatures are required to ensure a product that is safe for consumption.

Sensory evaluation would also be vital to further investigation of moisture enhancement/enzyme injection not only for the analysis of tenderness, as discussed in Section 5.3.2.2., but also to ensure palatability of the injected product. While many reports have documented the sensorial effects of injection on pork, Prestat et al. (2002) discussed that enhancement solutions may alter flavour notes differently in beef. Sutton et al. (1997) described a concise and effective approach to sensory evaluation for injected pork loins including measures of juiciness, tenderness, pork flavour, salt intensity, and alkalinity with standards provided for salt and alkalinity ratings to improve rating accuracy. Unstructured line scales gave panelists freedom from predetermined categories and red lights in the evaluation booths were used to mask obvious colour differences between injection treatments. This methodology could be easily adapted for beef evaluation.

As consumer acceptance of enhanced beef increases, so too does the significance of tenderness and palatability of such products, since these are the most important quality attributes (Ashie et al. 2002). Furthermore, although consumers are generally agreeable to purchasing enhanced products, concerns with added ingredients must be addressed (Brewer et al. 2002), perhaps with an effective education program to complement this type of product. Ultimately, consumers desire consistently palatable, safe, convenient, and affordable meat products (Kastner et al. 2001). With careful attention to phosphate and enzyme application combined with appropriate preparation techniques, this type of product can certainly be created from beef *Semitendinosus*.

5.5. Tables

Table 5.1. Summary of carcass characteristics of the 16 beef steers from which *Semitendinosus* muscles were harvested for the injection experiment.

Animal	Liveweight	Grade	Grade note[z]	pH_{24}[y]
1	457.5	n/a[x]		n/a[x]
2	536.0	B1	m	5.77
3	541.0	B4	c	6.03
4	554.0	B4	mc	5.96
5	531.5	B4	mc	5.54
6	582.5	A		5.55
7	493.0	B4	mc	5.81
8	450.0	B1	m	5.65
9	545.0	A		5.56
10	534.0	A		5.75
11	508.0	A		5.43
12	527.0	B4	c	5.87
13	512.5	A		5.48
14	521.5	A		5.51
15	528.0	A		5.61
16	465.0	B4	c	5.53

[z]Explanatory notes provided by grader to explain "B" grade classification, "m" indicating a deficiency in muscling and "c" a dark red colour

[y]Longissimus muscle pH at 24 h postmortem

[x]Grade and pH data not available for this carcass

Table 5.2. Brine components and proportions

		105% Injection level		110% Injection level	
Ingredient	**Target concentration in injected product (%)**	**Percent in brine[z]**	**Weight in brine (kg)[y]**	**Percent in brine**	**Weight in brine (kg)**
Salt	0.5	10.5	2.1	5.5	1.1
Tripolyphosphate	0.25	5.25	1.05	2.75	0.55
Pancreatin	0.01	0.21	0.042	0.11	0.022
Water[x]			16.808		18.328
Total volume (l)			20.00		20.00

[z]Percent in brine = (% target in product x % yield) ÷ % injection
[y]Weight in brine = (% in brine x volume of brine) ÷ 100
[x]Water determined by subtraction

Table 5.3. Effects of injection level and treatment on retained injection levels (%) and effect of injection treatment on weight loss (%) to 48 h post-injection.

	Retained injection level		Weight loss
Injection treatment[z]	**105%**	**110%**	
CON	n/a[y]	n/a	4.74a
ENZ	97.34	97.99	2.45b
ME	104.78	110.22	-6.89c
MEZ	104.93	110.85	-7.20c

[z]ENZ = pancreatin enzyme cocktail; ME = salt/phosphate brine; MEZ = combination salt/phosphate with pancreatin
[y]Not injected
a,b,c Weight loss values followed by different letters are significantly different ($P<0.05$)

Table 5.4. Effects of injection level and injection treatment on colour and pH of *Semitendinosus* measured at 48 h post-injection.

	Injection level (%)				Injection treatment[z]					
	105	**110**	**SEM**	**P**	**CON**	**ENZ**	**ME**	**MEZ**	**SEM**	**P**
pH	5.67	5.69	0.02	ns[y]	5.56a	5.59a	5.76b	5.80b	0.03	<0.01
L*	41.35	43.08	0.42	0.01	43.95a	47.15b	39.26c	38.51c	0.59	<0.01
Chroma	20.61	21.72	0.34	0.03	23.12a	23.67a	19.50b	18.38b	0.48	<0.01
Hue	25.65	25.93	0.29	ns	27.47a	28.53a	23.66b	23.48b	0.41	<0.01

[z]CON = control; ENZ = pancreatin enzyme cocktail; ME = salt/phosphate brine; MEZ = combination salt/phosphate with pancreatin

[y]Non-significant, P>0.05

a,b,c Within a treatment, values in the same row followed by a different letter are significantly different (P<0.05)

Table 5.5. Injection level, injection treatment, cooking temperature, and muscle location effects on cooking yield, Warner-Bratzler shear and shear standard deviation.

	Cooking yield (%)	Perpendicular shear[z] (kg)	Parallel shear[y] (kg)	Shear standard deviation[x]
Injection level				
105%	78.53	7.77	4.88	1.58
110%	74.07	7.13	4.44	1.17
P (SEM)	<0.01 (0.61)	0.01 (0.17)	0.02 (0.13)	<0.01 (0.09)
Injection treatment[w]				
CON	74.95a	7.52ab	4.95a	1.23a
ENZ	70.87b	7.52ab	4.87a	1.27a
ME	80.94c	7.91a	4.58ab	1.68b
MEZ	78.45d	6.87b	4.23b	1.31a
P (SEM)	<0.01 (0.86)	0.03 (0.25)	0.04 (0.19)	0.04 (0.12)
Cooking temperature				
72°C	79.27	7.84	4.80	1.57
77°C	73.33	7.07	4.51	1.21
P (SEM)	<0.01 (0.61)	<0.01 (0.17)	0.13 (0.13)	<0.01 (0.09)
Location[v]				
LD	76.07	6.48a	4.08a	1.29
RD	75.25	6.49a	4.10a	1.24
LP	76.79	8.64b	5.20b	1.47
RP	77.10	8.19b	5.26b	1.50
P (SEM)	0.43 (0.86)	<0.01 (0.24)	<0.01 (0.19)	0.36 (0.12)

[z]Shearing conducted perpendicular to fibre direction; [y]Shearing conducted parallel to fibre direction

[x]Standard deviation calculated across six samples within each steak

[w]CON = control; ENZ = pancreatin enzyme cocktail; ME = salt/phosphate brine;MEZ = combination salt/phosphate with pancreatin

[v]LD = left, distal; RD = right distal; LP = left proximal; RP = right proximal

a,b,c,d Values within a table section followed by a different letter are significantly different (P<0.05)

5.6. Figures

Figure 5.1. Schematic representation of the division of steaks shearing parallel to fibre direction indicating: initial strips cut parallel to the Y axis (**a**); creation of an "internal strip" on the newly exposed internal surface of the initial strip (**b**); final subdivision of internal strip, with cooked surfaces removed, to create cubes for Warner-Bratzler shear force measurement (**c**). Double ended arrows indicate cuts made to a 15 mm width at the various levels of division.

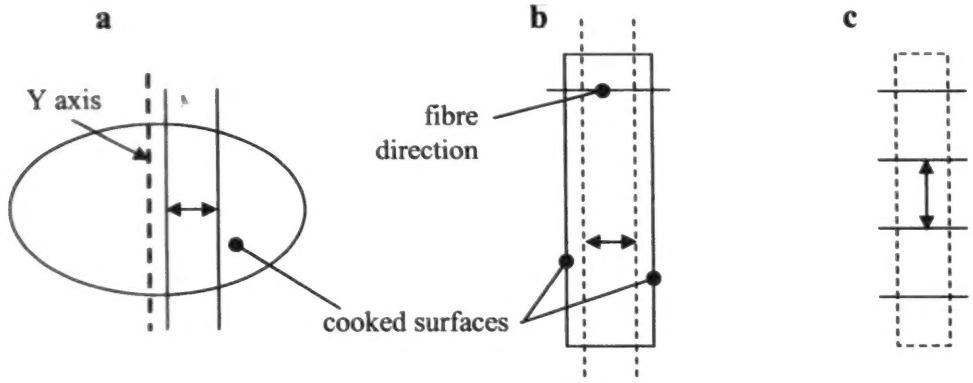
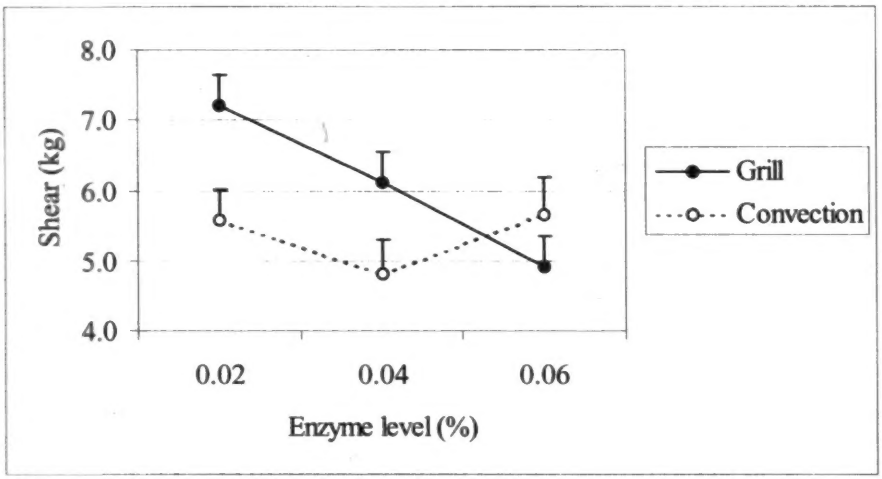


Figure 5.2. Interaction effect on Warner-Bratzler shear of enzyme concentration (0.2-0.6% in final product) and cooking method (electric grill vs. convection oven) at 105% injection. Vertical bars are standard errors.



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General Summary and Conclusions

The initial objective of this work was to develop a method for mapping Warner-Bratzler shear force, taking into account the shortcomings of previously reported methods as discussed in Chapter One. As such, a method was developed to thoroughly examine the *Longissimus thoracis et lumborum*, often the "benchmark" muscle in meat quality investigation, along its longitudinal, medial-lateral, and superficial-deep planes while maintaining reference to skeletal landmarks (Chapter Two). Avoiding an ageing period by examining muscles at 24 hours postmortem minimized postmortem proteolysis. Measures were also taken to equalize potential variability in muscle chilling rates; however in doing so, the likelihood of the occurrence of cold shortening was increased despite efforts to moderate carcass cooling conditions. The shear gradients observed were likely the result of variable internal tension development during the onset of rigor mortis in a muscle characterized by a complex array of origin and insertion points, although the trends were likely exaggerated by the occurrence cold induced muscle contraction.

With the mapping method in place, the next objective was to test its usefulness for examining the effects of an altered carcass suspension treatment on *Longissimus* shear trends and sarcomere length, a related tissue characteristic. The influence of such treatment on shear and sarcomere length is well documented in the literature. The results presented in Chapter Three highlight the usefulness of the mapping method for the examination of both raw and cooked samples from within the same muscle specimen. Again, spatial orientation was maintained in order to permit the examination of precise, individual locations within the muscle such that the effects of the carcass treatment could be documented in detail, rather than in general terms that resort to the use of average values for shear and sarcomere length.

An additional objective addressed in Chapter Three was the evaluation of both modified carcass chilling and extended postmortem ageing on several muscles in order to

determine the muscle specific effectiveness of these treatments. The variable response of the five muscle examined demonstrated the need for attention to specific muscle treatments in order to maximize tenderness while optimizing cooler space and time.

Use of the mapping method was then expanded to the *Semitendinosus* to determine its inherent shear gradients. Based on the fusiform fibre arrangement and knowledge about the contraction of connective tissue during cooking, a steak level intervention, the presence or absence of epimysial connective tissue, was examined to determine its effect on shear values via expulsion of water from the meat during heating. While the removal of the connective tissue layer reduced shear values, principal components analysis did not indicate a relationship between moisture content and shear. Furthermore, sarcomere length was poorly related to shear indicating that other factors were responsible for *Semitendinosus* toughness.

Based on results presented in previous chapters and in the literature, it appeared that more extreme measures were required to ameliorate toughness in the *Semitendinosus*. As such, a series of experiments involving the use of an injected enzyme complex, both with and without a phosphate-containing moisture enhancement brine, were completed. Preliminary work indicated that the enzyme treatment successfully met the shear reduction objective and further investigation showed that the inclusion of phosphate assisted with the process. Variable results, however, were a clear indication of the need for refinement of both the enzyme and moisture enhancement treatments for use under specific cooking conditions. Furthermore, the addition of sensory evaluation to the investigation would ensure the development of an acceptable palatable product and would perhaps yield more useful information about a moisture enhanced product than a simple shear force evaluation can provide.

The inevitable by-product of scientific investigation is the generation of a list of questions at least as long as that originally posed. The present work demonstrated the functionality of the mapping method for the investigation of not only Warner-Bratzler shear, but other related traits in either raw or cooked samples as well. It would be incredibly beneficial to apply this technique to the entire carcass musculature, and to muscles subjected to various conditions including ageing, altered suspension, electrical stimulation, and modified chilling regimes, in order to create three dimensional maps of

each muscle/treatment combination. Ultimately, the development of a database similar to that presented by Jones et al. (2001; full reference listed in Chapter Two) could provide a rapidly accessible reference to reveal the optimal use of any given muscle.

Correlation coefficients from analysis of the relationship of Warner-Bratzler shear values across various locations in the *Longissimus thoracis et lumborum*

Table A.1. Correlation coefficients (and P value) amongst longitudinal locations

	Central	Thoracic
Lumbar	<0.01 (0.96)	<0.01 (0.99)
Central		**-0.08** (0.04)

Table A.2. Correlation coefficients (and P value) amongst steaks within the lumbar region

	Steak 2	Steak 3	Steak 4	Steak 5
Steak 1	0.04 (0.59)	**0.24** (<0.01)	**0.23** (<0.01)	0.03 (0.67)
Steak 2		0.13 (0.06)	**0.20** (<0.01)	0.10 (0.15)
Steak 3			**0.25** (<0.01)	-0.09 (0.20)
Steak 4				0.05 (0.52)

Table A.3. Correlation coefficients (and P value) amongst steaks within the central region

	Steak 7	Steak 8	Steak 9	Steak 10
Steak 6	0.08 (0.23)	0.08 (0.22)	-0.05 (0.51)	0.13 (0.06)
Steak 7		**0.27** (<0.01)	0.12 (0.09)	0.12 (0.07)
Steak 8			**0.25** (<0.01)	0.07 (0.29)
Steak 9				0.13 (0.06)

Table A.4. Correlation coefficients (and P value) amongst steaks within the thoracic region

	Steak 12	Steak 13	Steak 14	Steak 15
Steak 11	0.12	0.10	**0.38**	**0.34**
	(0.16)	(0.25)	(<0.01)	(<0.01)
Steak 12		**0.32**	0.13	0.16
		(<0.01)	(0.14)	(0.08)
Steak 13			**0.38**	**0.18**
			(<0.01)	(0.05)
Steak 14				**0.41**
				(<0.01)

Table A.5. Correlation coefficients (and P value) amongst medial region across all longitudinal locations

	Central	Thoracic
Lumbar	0.27	0.06
	(0.09)	(0.71)
Central		0.07
		(0.46)

Table A.6. Correlation coefficients (and P value) amongst medial-central region across all longitudinal locations

	Central	Thoracic
Lumbar	-0.04	-0.06
	(0.72)	(0.58)
Central		**0.24**
		(<0.01)

Table A.7. Correlation coefficients (and P value) amongst lateral-central region across all longitudinal locations

	Central	Thoracic
Lumbar	0.08	-0.13
	(0.46)	(0.21)
Central		-0.10
		(0.23)

Table A.8. Correlation coefficients (and P value) amongst lateral region across all longitudinal locations

	Central	Thoracic
Lumbar	0.07	**-0.33**
	(0.55)	(0.02)
Central		-0.27
		(0.05)

Table A.9. Correlation coefficients (and P value) amongst medial-lateral zones within the lumbar region

	2	3	4	5	6	7	8	9	10
1	0.01	0.17	0.15	-0.04	0.27	0.14	0.25	0.06	0.01
	(0.96)	(0.27)	(0.33)	(0.80)	(0.08)	(0.38)	(0.88)	(0.71)	(0.94)
2		-0.05	-0.11	-0.04	0.03	0.11	-0.03	-0.01	0.07
		(0.60)	(0.27)	(0.68)	(0.81)	(0.29)	(0.76)	(0.90)	(0.60)
3			0.10	<-0.01	-0.03	-0.15	0.04	-0.15	-0.07
			(0.28)	(1.00)	(0.77)	(0.11)	(0.67)	(0.18)	(0.60)
4				<-0.01	-0.09	0.10	0.01	0.17	0.11
				(0.98)	(0.36)	(0.28)	(0.92)	(0.13)	(0.38)
5					**0.24**	0.18	**-0.22**	**0.30**	0.16
					(0.02)	(0.09)	(0.04)	(0.01)	(0.18)
6						0.17	0.09	0.09	0.09
						(0.10)	(0.41)	(0.42)	(0.94)
7							0.15	0.02	0.17
							(0.10)	(0.87)	(0.16)
8								0.07	-0.01
								(0.51)	(0.91)
9									**0.40**
									(<0.01)

Table A.10. Correlation coefficients (and P value) amongst medial-lateral zones within the central region

	2	3	4	5	6	7	8
1	-0.01	-0.02	-0.02	-0.03	0.07	**-0.23**	0.11
	(0.90)	(0.85)	(0.85)	(0.78)	(0.43)	(0.01)	(0.35)
2		**0.24**	0.08	-0.15	0.10	**0.34**	-0.12
		(<0.01)	(0.32)	(0.07)	(0.22)	(<0.01)	(0.31)
3			-0.13	0.13	0.12	0.03	0.02
			(0.12)	(0.11)	(0.14)	(0.75)	(0.87)
4				0.10	**0.24**	0.12	0.18
				(0.21)	(<0.01)	(0.14)	(0.13)
5					<0.01	**-0.27**	<-0.01
					(0.97)	(<0.01)	(0.98)
6						0.13	-0.04
						(0.12)	(0.73)
7							<-0.01
							(0.94)

Table A.11. Correlation coefficients (and P value) amongst medial-lateral zones within the thoracic region

	2	3	4	5
1	0.03	0.02	0.13	0.25
	(0.73)	(0.83)	(0.14)	(0.08)
2		**0.38**	0.13	0.09
		(<0.01)	(0.12)	(0.53)
3			**0.20**	-0.03
			(0.02)	(0.81)
4				0.15
				(0.28)

Appendix B

Experiment One: Correlation coefficients from analysis of the relationship of Warner-Bratzler shear values across various locations in *Semitendinosus*

Table B.1. Correlation coefficients (and P value) amongst steaks in the *Semitendinosus*

	2	3	4	5	6	7
1	0.34	0.37	-0.08	-0.14	0.04	**-0.58**
	(0.23)	(0.22)	(0.79)	(0.64)	(0.89)	(0.04)
2		0.04	0.49	**0.58**	**0.70**	0.06
		(0.89)	(0.06)	(0.02)	(<0.01)	(0.82)
3			0.40	0.35	0.38	0.24
			(0.14)	(0.20)	(0.16)	(0.39)
4				**0.94**	**0.92**	**0.61**
				(<0.01)	(<0.01)	(0.02)
5					**0.95**	**0.54**
					(<0.01)	(0.04)
6						0.46
						(0.09)

Table B.2. Correlation coefficients (and P value) amongst shear sample locations in the *Semitendinosus*

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0.31 (0.61)	0.47 (0.42)	-0.34 (0.57)	0.71 (0.18)	0.40 (0.50)	0.62 (0.27)	0.65 (0.27)	0.58 (0.30)	0.52 (0.37)	0.37 (0.55)	0.47 (0.43)	**-0.93** (0.02)	0.45 (0.44)	0.31 (0.61)	0.23 (0.71)
2		0.51 (0.24)	0.99 (0.13)	**0.76** (0.05)	0.69 (0.09)	0.55 (0.20)	0.38 (0.40)	0.57 (0.18)	0.40 (0.38)	0.49 (0.26)	0.57 (0.18)	-0.27 (0.66)	0.58 (0.17)	0.51 (0.24)	0.47 (0.29)
3			-0.25 (0.64)	0.63 (0.13)	0.75 (0.05)	**0.90** (0.01)	0.66 (0.11)	0.75 (0.05)	**0.84** (0.02)	**0.92** (<0.01)	**0.87** (0.01)	-0.17 (0.78)	**0.83** (0.02)	**0.93** (<0.01)	**0.86** (0.01)
4				0.34 (0.51)	0.16 (0.77)	-0.18 (0.73)	-0.11 (0.84)	-0.06 (0.92)	-0.30 (0.56)	-0.22 (0.68)	-0.11 (0.83)	0.07 (0.91)	-0.03 (0.96)	-0.20 (0.71)	-0.20 (0.71)
5					0.47 (0.29)	0.72 (0.07)	0.71 (0.08)	0.41 (0.36)	0.25 (0.59)	0.38 (0.40)	0.44 (0.33)	-0.45 (0.44)	0.46 (0.30)	0.44 (0.33)	0.37 (0.41)
6						0.72 (0.07)	0.57 (0.19)	**0.90** (0.01)	**0.80** (0.03)	**0.82** (0.02)	**0.90** (<0.01)	-0.32 (0.60)	0.70 (0.08)	0.73 (0.06)	0.67 (0.10)
7							0.60 (0.16)	**0.84** (0.02)	**0.79** (0.03)	**0.83** (0.02)	**0.88** (0.01)	-0.36 (0.55)	0.59 (0.16)	**0.84** (0.02)	**0.82** (0.03)
8								0.39 (0.39)	0.31 (0.50)	0.41 (0.37)	0.42 (0.35)	-0.44 (0.46)	0.54 (0.21)	0.36 (0.43)	0.21 (0.65)
9									**0.91** (<0.01)	**0.86** (0.01)	**0.97** (<0.01)	-0.47 (0.42)	0.60 (0.16)	**0.80** (0.03)	0.79 (0.03)
10										**0.97** (<0.01)	**0.96** (<0.01)	-0.31 (0.61)	0.74 (0.06)	**0.93** (<0.01)	**0.91** (<0.01)
11											**0.96** (<0.01)	-0.12 (0.85)	**0.81** (0.03)	**0.98** (<0.01)	**0.95** (<0.01)
12												-0.28 (0.64)	0.72 (0.07)	**0.93** (<0.01)	**0.90** (<0.01)
13													-0.23 (0.71)	-0.04 (0.95)	0.03 (0.96)
14														**0.80** (0.03)	0.69 (0.09)
15															**0.98** (<0.01)

Experiment Two: Correlation coefficients from analysis of the relationship of Warner-Bratzler shear and sarcomere length across various locations in the *Semitendinosus*

Table B.3. Correlation coefficients (and P value) within distal, mid, and proximal sections

Section	R	P
Distal	-0.22	0.22
Mid	-0.09	0.64
Proximal	-0.27	0.14

Table B.4. Correlation coefficients (and P value) within quadrants

Quadrant	R	P
1	**0.45**	0.02
2	0.09	0.68
3	0.02	0.93
4	-0.02	0.94